STUDIES ON THE BAR SERIES OF DROSOPHILA MELANOGASTER. II. THE TEMPERATURE-EFFECTIVE PERIOD FOR FACET DETERMINATION IN BAR AND BAR VESTIGIAL

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In an earlier paper of this series (MARGOLIS 1935), it was shown that the gene *vg* effected a small but significant decrease in facet numbers in Bar-eyed flies of Drosophila. The present paper deals with the results of an investigation of the temperature-effective period for facet determination in *B* and *B vg* stocks. The early investigations of SEYSTER (1919) and KRAFKA (1920) served to localize the temperature effect on facet number entirely within the larval period of development. More recently, DRIVER (1926, 1931) and LUCE (1931) succeeded in setting more definite limits for the T.E.P. in *B* and B^i flies at different temperatures. DRIVER, especially, has called attention to the necessity of precision in determining temperature-effective periods as a tool in analyzing the kinetics of facet determination in the Bar series. The results of these investigators will be discussed in greater detail in relation to the results obtained by the writer.

For expediency, the temperature-effective period for facet determination will be referred to as the T.E.P.

EXPERIMENTAL

The method employed in T.E.P. studies consists in transferring flies from one temperature to another at successive intervals throughout a portion of larval development. The transferred individuals then complete their development at the second temperature. Facet counts are made on the adult flies in the various transfer groups.

The stocks used and the general methods employed in this investigation have been discussed fully in the paper previously cited. Since a short egg-laying period is desirable in an investigation of this type, 20 to 25 pairs of flies were permitted to lay eggs for a 1-hour period. Five to 7 successive batches of eggs were obtained from each group of parents. In a few cases, where an insufficient number of eggs had been laid, a 1.5 hour egg-laying period was used. Egg-laying took place at room temperature (c. 22°C). The experimental vials were then immediately placed at 28°C to begin their development. Beginning at about the 40th hour from the midpoint of the egg-laying period, a suitable number of cultures were transferred at intervals to 22° to complete their development. The experiment was so arranged that each transfer group covered a range of 5 to 7 hours, at one

hour intervals. The total range of ages of larvae at time of transfer was from 40 to 80 hours. *B* and *B vg* flies were reared at the same time, under identical culture conditions, and in the same thermostats. In order to accelerate the attainment of the new temperature equilibrium at transfer,

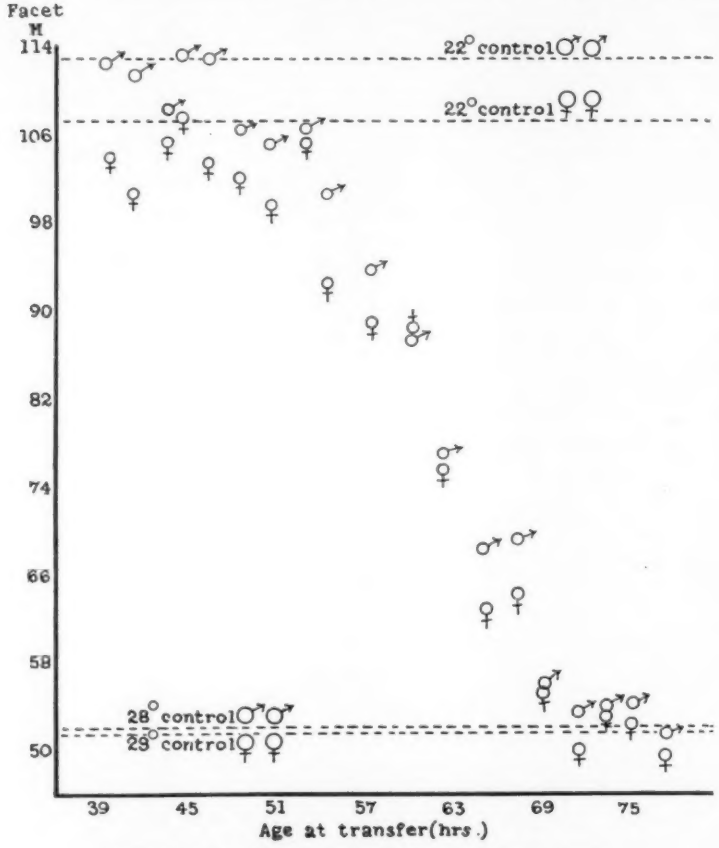


FIGURE 1.—Mean facet numbers in *B* flies transferred at successive intervals from 28° to 22°.

a water bath a few tenths of a degree below 22° was used. By this method only 7 to 8 minutes were required for temperature equilibrium to ensue.

Due to low fertility and viability, very few flies were obtained from some of the transfer groups. It was found necessary, in some cases, to combine the data of two or three successive transfer groups in order to reduce sampling errors in the facet counts. When this was done, a mean time of

transfer was calculated. The data on the T.E.P. are presented in tables 1 and 2 and in figures 1 and 2. Males and females have been recorded separately, both in the tables and the figures, because of sexual dimorphism in facet number. Although the points in the figures show a fairly large scatter, it is apparent that the general trend is sigmoid in character. Most investigations on the T.E.P. for facet determination, as

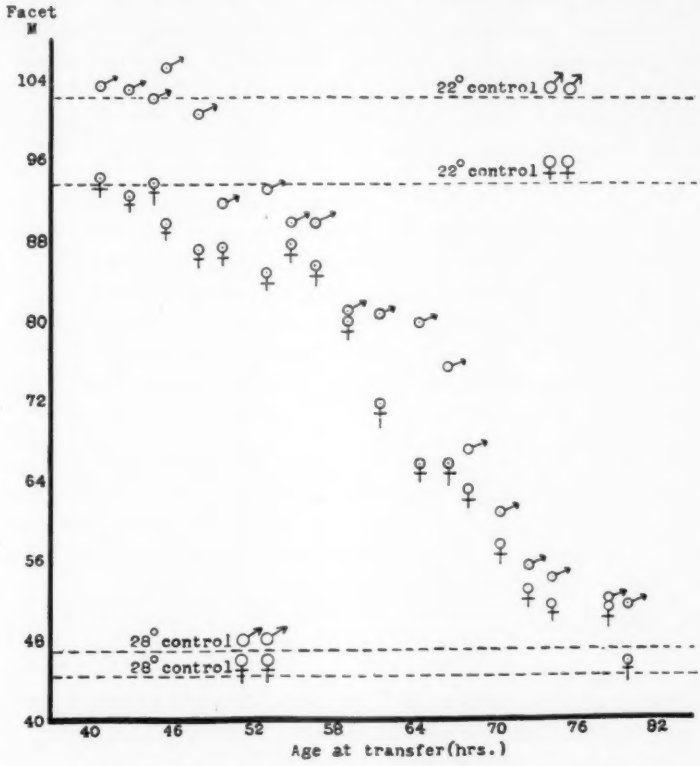


FIGURE 2.—Mean facet numbers in *B vg* flies transferred at successive intervals from 28° to 22°.

well as those for other characters show this relation. The significance of this point will be discussed later.

In establishing the limits of the T.E.P. a method frequently used is to take the first point at each end of the curve which shows a statistically significant deviation from the control line. For example, in the case of the *B* males, the first significant deviation from the 22° control line is at

48.5 hours. Since the beginning of the T.E.P. must be somewhat earlier, the mean between 48.5 hours and the point preceding it (46.5 hours) is taken as marking the beginning of the T.E.P. Similarly, the end of the T.E.P. may be found. Since there is very little difference between males and females in the duration of the T.E.P., the data for the two sexes are here taken together. There is, however, some slight indication that the females may enter the period a little earlier. Delimiting the T.E.P. as indicated above, we find that in *B* the period begins at 48.5 hours and ends at 74.5 hours; in *B vg* the period begins at 47.6 hours and ends at 76.2 hours. These values, although by no means conclusive, indicate that the T.E.P. may be slightly longer in *B vg* than in *B*. It will be shown later that this method of determining the period involves considerable error.

A somewhat better method for establishing the duration of the T.E.P. is based upon the trend of the points. We may take the first point showing the beginning of the trend away from the 22° control line as a marker. Taking, as before, the mean of this point and the one preceding it, we consider the value so obtained as the beginning of the T.E.P. Similarly, the end of the T.E.P. may be found. By this method, it may be seen from figures 1 and 2 that the period in *B* begins at 46.6 hours and ends at 76.6 hours; in *B vg* the period begins at 45.7 hours and ends some time after 80 hours. It appears that the method of trends gives a more accurate value of the limits of the T.E.P. than does the estimation based upon statistically significant deviations. When sampling errors are relatively large, as is the case in these data, real individual differences may be obscured. The general trend of the points is affected to a lesser extent, and consequently gives more accurate results.

The values for the duration of the T.E.P., as determined by either of the methods considered above, have little meaning, except qualitatively. In establishing the limits of the T.E.P. we have really determined the time at which the first few individuals of the population enter their respective T.E.P.'s, and the time at which the last few individuals emerge. This will represent the mean value of the T.E.P. of all the individuals only in the limiting case when the temporal position of the T.E.P. in relation to the whole period of development is the same in all individuals, that is, when every individual enters the T.E.P. at the same time, and emerges at the same time. We should certainly not expect this to be actually true. From tables 1 and 2 we see that the coefficients of variability for transfer groups which have entered the T.E.P. show a marked increase. As soon as the T.E.P. is completed the value falls to the average level. This is true in *B*, but not in *B vg*, indicating that the latter had not completed the T.E.P. Clearly then, some factor is contributing to this additional variability in

TABLE 1

Temperature-effective period (28° to 22°) Bar.

HRS. AT 28° BEFORE TRANSFER	MALES M	MALES PERCENT V	MALES n	FEMALES M	FEMALES PERCENT V	FEMALES n
0.0	112.9±1.6	17.5	155	107.1±1.4	14.5	118
39.5	112.3±3.3	12.7	20	103.8±4.5	16.3	14
41.3	111.2±2.9	16.7	42	100.5±2.4	14.4	37
43.7	108.0±2.3	18.7	79	105.2±2.9	17.6	41
44.8	113.2±1.9	15.5	83	107.4±1.8	13.7	66
46.5	112.7±2.7	18.4	59	103.3±2.7	16.7	42
48.5	106.2±2.7	19.7	57	102.0±3.3	17.6	30
50.8	105.0±2.1	18.6	84	99.4±1.9	16.6	73
53.0	106.5±3.4	17.4	29	105.2±2.8	13.6	27
54.5	100.4±2.2	17.9	69	92.5±1.8	15.5	64
57.7	93.5±2.5	17.6	45	88.7±2.6	18.7	42
60.3	87.1±2.1	21.8	84	88.3±2.6	22.3	57
62.3	77.0±2.1	23.5	75	75.5±2.3	20.7	45
65.1	68.4±2.3	24.0	51	62.7±1.7	16.3	36
67.4	69.1±2.0	24.5	70	64.2±1.4	15.6	54
69.3	56.0±1.2	17.1	65	55.4±1.6	18.8	41
71.8	53.5±0.9	13.1	61	50.0±0.7	10.4	60
73.6	54.1±0.6	9.3	60	53.3±0.7	10.7	65
75.4	54.3±1.2	12.3	29	52.5±0.7	9.3	51
77.8	51.5±0.7	10.9	64	49.5±0.6	8.1	39
Total time	52.1±0.4	10.0	149	51.6±0.4	9.3	122

TABLE 2

Temperature-effective period (28° to 22°) B vg.

HRS. AT 28° BEFORE TRANSFER	MALES M	MALES PERCENT V	MALES n	FEMALES M	FEMALES PERCENT V	FEMALES n
0.0	102.1±1.9	15.3	66	93.4±1.9	16.1	64
40.5	103.3±3.7	15.7	19	93.9±2.2	15.4	45
42.6	102.8±3.1	21.8	53	92.2±2.1	13.0	31
44.4	102.0±2.7	16.7	38	93.5±2.9	16.0	27
45.2	105.0±4.5	19.5	20	89.5±2.2	11.3	21
47.7	100.3±2.7	17.4	42	87.0±1.8	13.2	39
49.6	91.5±1.9	14.6	50	87.3±1.8	12.1	33
52.8	92.9±2.5	18.8	51	84.7±2.0	15.5	41
54.7	89.6±2.2	18.6	60	87.5±2.2	14.3	32
56.4	89.6±2.6	20.6	51	85.4±2.2	15.8	39
58.9	80.7±1.7	13.3	38	79.8±2.0	12.9	25
61.4	80.5±3.8	27.3	34	71.5±3.5	19.3	16
64.2	79.7±3.5	27.1	37	65.5±2.9	23.4	28
66.3	75.2±2.2	23.3	61	65.7±1.7	20.5	62
68.0	67.0±3.9	30.3	27	63.0±4.4	31.9	21
70.4	60.6±2.5	24.6	36	57.5±2.4	23.0	30
72.4	55.4±1.5	16.9	37	53.1±2.3	22.6	28
74.2	54.2±2.0	19.2	28	51.5±2.1	22.3	30
78.3	52.0±3.3	30.8	23	51.3±3.6	24.9	13
80.0	51.5±3.3	23.9	14	45.8±3.2	31.2	20
Total time	47.0±1.0	13.6	38	44.5±0.7	8.1	25

populations which have entered the T.E.P. If individuals of any one transfer group enter their respective T.E.P.'s at different times, an increase in the coefficient of variability is to be expected, since the transfer group is then a heterogeneous population in respect to the temperature effect on facet number. It may be demonstrated that this is actually the case.

Since flies show variability in the length of the egg-larval period, we may segregate individuals at the time of puparium formation, and determine the correlation coefficient between facet number and length of egg-larval period. This was done at several temperatures. The data are presented in table 3. With the exception of females at 22.7°, no significant

TABLE 3

Correlation between length of egg-larval period (x) and facet number (y).

BAR

T	r	M_x	M_y
	MALES		
22.7°	−0.049±0.088	135.0±0.48	95.4±1.40
27.0°	−0.193±0.130	97.7±0.50	58.1±0.88
28.0°	−0.142±0.137	96.2±0.54	49.9±0.81
29.0°	0.020±0.143	96.5±0.71	41.8±0.53
	FEMALES		
22.7°	−0.240±0.077	134.3±0.50	93.6±1.02
27.0°	−0.009±0.147	97.2±0.53	55.2±0.94
28.0°	−0.212±0.155	96.3±0.85	48.4±1.00
29.0°	−0.101±0.162	95.8±0.92	40.1±0.59

correlation was found. It should be noted, however, that with a single exception (males at 29.0°) all the values of r are negative, although not significant statistically. The writer has found that under crowded culture conditions there is a significant negative correlation between facet number and length of the egg-larval period. Since crowding tends to increase the time of development, this result indicates that those flies which are delayed the most show the smallest facet counts. The fact that the values of r in table 3 are consistently negative may indicate slight crowding effects in the cultures, which are too small to manifest themselves in the mean facet counts. In relating these facts to the problem of the T.E.P. we have the following considerations. We should in general expect those individuals which complete the egg-larval period first, to have completed other developmental phases relatively earlier than those individuals which develop more slowly. If this is true then those individuals in the transfer groups which pupate first will have passed a longer portion of their T.E.P.'s at 28° than those pupating later, and will consequently have smaller facet numbers. This relation is the converse of that found in flies raised at a single temperature under crowded conditions.

This point was tested by determining the correlation coefficient between

facet number and length of egg-larval period for a group of *B* flies transferred from 28° to 22° at approximately the middle of the T.E.P. The values obtained for the correlation coefficients are:

$$\text{males} = 0.27 \pm 0.071 \ (n = 172)$$
$$\text{females} = 0.28 \pm 0.066 \ (n = 197)$$

These values are positive and statistically significant and show that different individuals of the same age as expressed in hours of development, are in different stages of their individual T.E.P.'s.

The T.E.P. which we actually determine experimentally is then composite of two quantities, (1) the mean length of the T.E.P.'s of the individuals, and (2) the mean range of variation in time at which the T.E.P.'s of the individuals occur. For convenience, in the following we shall adopt the terminology used by CHILD (1935) who studied the T.E.P. for bristle determination in scute.

Let P = the length of the T.E.P. of the whole population as determined experimentally (apparent T.E.P.)
D = the mean length of the individual T.E.P.'s
V = the mean range of variation between the midpoints of the individual T.E.P.'s (D)

From the conditions set down it is apparent that we have the following relation: $P = D + V$. It is apparent that several possible relations may subsist between D and V. The value of V may equal O, so that $P = D$. We have already seen that this is not the case, since a positive correlation between facet number and length of the egg-larval period is obtained in the transfer groups. The value of D may equal O, so that $P = V$. If this were true the facet distributions in all the transfer groups would be bimodal, especially since the two distributions do not overlap. Those flies which had not yet been affected by temperature at the time of transfer would show the 22° facet distribution, while those which had been affected would show the 28° distribution. Since none of the transfer groups shows definite bimodality, this possibility may also be dismissed. We are left then with the third possibility that D and V both have finite values.

The problem with which we are concerned is the evaluation of D, in order to ascertain whether *vg* has any effect on the duration of the T.E.P. for facet determination in the individual. This problem will be considered fully in a later section.

Duration of Egg-Larval Period in Transfer Groups

The length of the egg-larval period was determined in a number of the groups transferred from 28° to 22° at intervals during the larval period. LUDWIG (1933) has shown that when Drosophila pupae are transferred back and forth between two temperatures, the effects of each temperature

on length of the pupal period are, in general, additive. The data of table 4 give the mean values for length of the egg-larval period for different transfer groups. No attempt was made to keep separate records for males and females since earlier data showed no sex difference. The calculated values are based on the assumption that the effects of the two temperatures are additive. In general there is a fairly good agreement between the observed and calculated values. This agreement is apparently much better for *B vg* than for *B*. The fact that the effects of the two temperatures on length of the egg-larval period are additive, shows that no effect of the first temperature is carried over to development at the second temperature after the flies are transferred.

TABLE 4

Duration of egg-larval period in flies transferred from 28° C to 22° C.

HRS. AT 28° BEFORE TRANSFER	*B*		*B vg*	
	M (obs.)	M (calc.)	M (obs.)	M (calc.)
0.0	143.1±0.26	..	150.3±0.98	..
40.9	120.4±0.55	122.8	129.8±1.70	129.0
45.8	117.0±0.56	120.2	..	..
48.0	..	..	125.6±1.08	125.4
59.8	111.0±0.56	113.3	..	..
61.8	109.4±0.64	112.3	..	..
65.0	..	..	115.2±1.05	116.6
66.8	109.4±0.39	109.7	..	..
70.0	..	..	113.0±0.96	114.0
72.9	108.0±0.45	106.7	113.6±1.56	112.5
73.9	104.2±0.52	106.3	112.6±1.23	112.0
75.8	105.0±0.44	105.2	..	..
77.9	100.0±0.66	104.1	109.8±1.30	110.0
79.9	102.2±1.0	103.3	108.8±1.24	109.0
Total time	95.4±0.19	..	98.9±0.51	..

THEORETICAL

Analysis of the temperature-effective period

It has been shown that the T.E.P. for the population as a whole (P) is composite of two factors:

(1) the mean length of the individual T.E.P.'s (D), and

(2) the mean range of variation of the midpoints of the D's (V).

Since $P = D + V$,

it is clear that if we can evaluate V, we can readily determine D, assuming that the value of P, as experimentally determined, is correct. It was thought that V could be evaluated from a comparison of the facet distributions in the transfer groups with those of the controls at 22° and 28°. A consideration of the manner in which the facet frequency distributions in the transfer groups are built up will illustrate the nature of the problem.

Let us assume that we know the form of the distribution of times at which various fractions of the population enter the T.E.P., and that a fraction a_1, enters at t_1, a_2 enters at t_2, a_n enters at t_n. We shall assume, moreover, that the time-facet function is known for 28° and 22°. By time-facet function, as used here, is meant the relation describing the course of the facet-forming processes in time. If now, we transfer the population from 28° to 22° at time t_x—and for simplicity we shall assume that at time t_x the whole population is actually in the T.E.P.—we observe the following condition of the population in respect to facet formation at the completion of development. A fraction of the population a_1 will have formed x_1 facets at 28° and the remainder at 22°; a_2 will have formed x_2 facets at 28° and the remainder at 22°; a_n will have formed x_n facets at 28° and the remainder at 22°. At the completion of development each fraction of the transfer group, $a_1, a_2, \cdots a_n$, will show a mean facet number and a particular facet distribution. The mean facet number for any fraction of the population ($a_1, a_2, \cdots a_n$) is uniquely defined by the position of that fraction on the facet axis of the time-facet curve at 28° and 22° at the time of transfer. We shall assume that the actual facet distribution for any fraction ($a_1, a_2, \cdots a_n$) of the population having a given mean is known. We may then very easily calculate the mean facet number and facet distribution for the whole group transferred at t_x. Similarly we may find the mean facet number and facet distribution for a group transferred at any time t. Plotting the means obtained in this way against the time of transfer we get a curve in which each point is descriptive of the relative numbers of facets formed at 28° and 22°, respectively, for the population as a whole. It is this relation which, in fact, has been obtained experimentally for *B* and *B vg*, respectively (figures 1 and 2).

The problem with which we are concerned at present, however, is not in building up the frequency distributions of the transfer groups and determining their moments, but rather in breaking them down into their components, thereby obtaining values for V and D. Having seen that a knowledge of the time-facet function and of the variation in time of the individual T.E.P.'s is essential to building up these distributions analytically, it is clear that without independent information concerning one or the other of these factors, no rigorous solution can be had. We may, however, through simplifying assumptions make some estimate of the number of individuals entering and emerging from the T.E.P. at different times. Plotting the percentage of the population in a transfer group which has entered the T.E.P. for each transfer group, we may obtain a curve which is really the integral of the frequency distribution of the beginning of the T.E.P. in the population.

Since those groups which have been exposed to 28° for progressively

longer periods of time will tend more toward the 28° distribution, we find in successive transfer groups greater proportions of the population at the low end of the distribution. Taking the 22° control median facet number as point of reference, we find in successive transfer groups that increasing numbers of individuals assume values less than the median at 22°. Let x = the percentage of the population of a transfer group having facet numbers less than the median value of the 22° control. Since in the control 50 percent of the population have values less than the median, (x percent − 50 percent) = excess percentage of individuals in the transfer group having values less than the median. These individuals clearly are ones which have entered the T.E.P. In addition, an equivalent proportion (x percent − 50 percent) of the transfer group which, if unaffected by the 28° temperature, would have developed facet numbers less than the 22° median, will also have entered the T.E.P. Hence 2(x percent − 50 percent) of the transfer group have entered the T.E.P. This method involves the assumption that any individual which enters the T.E.P. is immediately shifted a sufficient amount along the facet axis of the time-facet curve to cross the median value of the 22° controls. This assumption is very probably incorrect, and consequently this method gives, in any transfer group, a *minimum* number of individuals which have entered the T.E.P. By a similar method we may estimate the proportion of the population of a transfer group which has emerged from the T.E.P. Taking the median value of the 28° facet distribution as a point of reference, we shall assume that only individuals which have emerged from the T.E.P. will show smaller facet numbers. If these represent x percent of a transfer group, then, since an equal proportion of flies having facet numbers greater than the 28° median will also have completed the T.E.P., the total proportion emerged will equal 2x percent. Since, of course, some flies which had not yet completed the T.E.P. at the time of transfer may have facet numbers less than the median of the 28° control, the value of x calculated in this way gives a *maximum*.

The percentage of individuals in different transfer groups which have entered into, and emerged from, the T.E.P. was calculated in the manner described above, and the relation is shown in figure 3 for *B* and *B vg*, respectively.

The 50 percent points on the curves in figure 3 represent the mean time of beginning and end of the T.E.P. in the two stocks under consideration. These mean values were calculated directly from the data before drawing the curves. The curves were then fitted to the points by eye in such a manner as to pass through these means and at the same time give a good fit to the largest number of points in each series. Originally separate

calculations were made for males and females for each point. Since these values showed a random scatter about a single curve, they were combined to give a weighted mean for the two sexes. The vertical lines through the points in the figure represent the magnitude of plus or minus twice the standard errors of the points. These errors were calculated from the expression: $E = \sqrt{\frac{pq}{n}}$, where p represents the chance of an individual entering or emerging from the T.E.P. at a particular time; $q = (1-p)$;

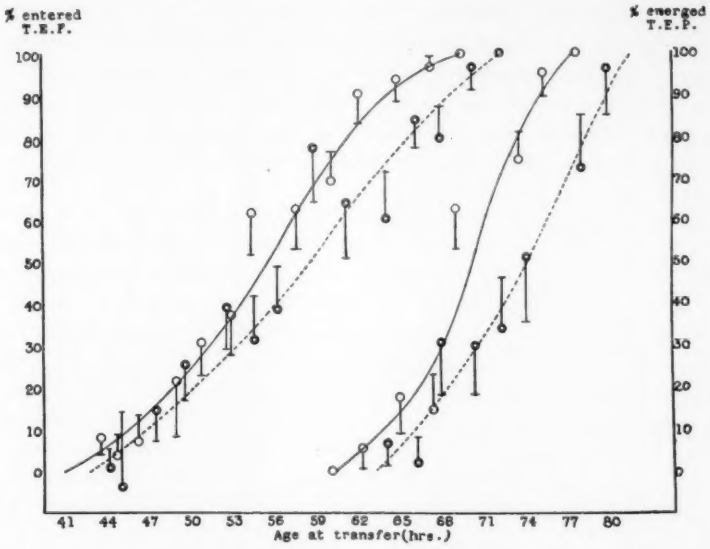


FIGURE 3.—Probability integrals for individual variability in time of occurrence of beginning and end of T.E.P.
Left-hand curves represent beginning of T.E.P.
Right-hand curves represent end of T.E.P.
Solid line = *B*; broken line = *B vg*.

and n is the size of the population. The value of p for each point was taken directly from the appropriate curve.

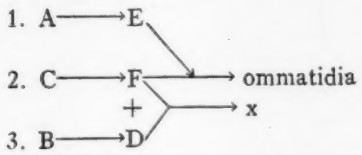
We have seen that the assumptions leading to the calculation of the points in figure 3 give *minima* for the points representing the percentage of flies which have entered the T.E.P. and *maxima* for the percentage of flies emerged. This means that the true integral curves for the distribution of the beginning of the T.E.P. will lie to the left of the calculated curves. Conversely, the true integral curves for the distribution of the end of the T.E.P. will lie to the right of the calculated curves. The difference between the 50 percent points in the two sets of curves therefore gives a *minimum*

value for D, the mean length of the T.E.P. in the individual. This value is 15.0 hours for *B* and 15.6 hours for *B vg*. The difference between the two values, 0.6 hours although quite small is very probably significant, especially since the 50 percent points on the curves represent means calculated from all the points and consequently have very small sampling errors. Moreover, a much greater increase in the duration of the T.E.P. in *B vg* is hardly to be expected since *vg* increases the total duration of larval life at 28° by only 2.4 hours (MARGOLIS 1935). We have seen that the assumptions leading to the calculation for the points in figure 3 give minima for the values of D for both *B* and *B vg*. It should be pointed out, however, that the difference between the values of D in the two stocks is not a minimum, but represents very probably a correct value within the limits of experimental and sampling error. This follows from the fact that the errors introduced by the assumptions inherent in the method of calculation affect the probability integral curves for the two stocks in the same way, if *vg* affects facet number in *B* by prolonging the duration of the T.E.P. and not by altering the time-course of facet determination. Although the data cannot conclusively establish the absence of any effect of *vg* on the form of the time-facet function, they do demonstrate an effect on the duration of the T.E.P. Inspection of the curves in figure 3 shows that the effect of *vg* in increasing the length of the T.E.P. occurs primarily in delaying the end of the period. Although it appears that *vg* does to some extent retard the beginning of the T.E.P., the effect on the end point is greater. This fact is confirmed by the results obtained on the "apparent" T.E.P. (P) estimated by the method of trends, and is consistent with the interpretation of the effect of *vg* on facet number in *B* presented in an earlier paper.

An estimate of P, the "apparent" T.E.P., may also be obtained from figure 3. This is done simply by determining the distance from the 0 percent points on the left-hand curves to the 100 percent points on the right-hand curves along the abscissa, for *B* and *B vg*, respectively. These values of P are 36.6 hours for *B* and 39.0 hours for *B vg*. Apart from any consideration of errors of extrapolation the value of P as estimated above is a minimum, theoretically, because of the assumptions inherent in the method of calculating the points for the curves. It can be demonstrated, however, that these assumptions affect the first flies to enter the T.E.P. and the last to emerge to only a very slight extent, and consequently introduce no great error in the above calculation. The fact that P as calculated above, although a theoretical minimum, is much greater than P calculated either by the trends of the points in figures 1 and 2, or by significant deviations from the control means, gives us some idea of the magnitude of the errors incurred by either of these methods.

It may be seen from figure 3 that the curves for the beginning of the

T.E.P. for both stocks show a much larger range of variation than the curves for the termination of the T.E.P. This difference in the two sets of curves seems too large to be fortuitous and must be accounted for by the factors which operate to determine the beginning and end of the T.E.P., respectively. For this purpose it is necessary to review briefly the mechanism postulated by the writer to explain the kinetics of facet determination in the Bar series. The following diagram is a schematic representation of the processes concerned.



Reaction 2 represents the facet-forming processes in the wild-type resulting in the production of F, whose quantity at a particular time determines the number of ommatidia. These processes also operate in *B* stocks. Reaction 3 represents the processes initiated by the Bar gene. These result in the production of D, a substance which either combines with F to form X, or catalyzes the conversion of F to X. Reaction 1 represents the general processes of development which determine the time at which the region containing F is converted into ommatidia.

The time limits of the T.E.P. have been identified with the beginning and end of the reaction F+D→X. It was assumed that F is formed at some early period in development so that the reaction F+D→X begins as D is being formed. The reaction stops when F is converted into ommatidia. The latter is governed by reaction 1, when the quantity of E attains a certain level. If these relations presented as a formal explanation of the facts known in the Bar series are essentially correct, we may readily account for the difference in variability in the beginning and end of the T.E.P. shown in figure 3. The beginning of the T.E.P. is determined by reaction 3 and represents a mutant process. The termination of the T.E.P., on the other hand, is governed by reaction 1, representing a group of wild-type processes. PLUNKETT (1932) and MULLER (1932) have pointed out that quite generally mutants show a much wider range of variability than the wild-type. The results obtained here are entirely consistent with this view.

CHILD (1935) in studying the T.E.P. for bristle determination for various bristles in scute-1, has found that the T.E.P. for the individual (D) is much shorter than the period determined experimentally (P). CHILD'S data show far more strikingly than those of the writer that individual variability in the temporal position of the T.E.P. is a large

source of error in experimental determinations of the T.E.P.

In earlier investigations on the T.E.P. in the Bar series, variability in the temporal position of the T.E.P. in different individuals was not taken into consideration in the analysis of the data. The T.E.P. as measured by other investigators consequently represents the "apparent" T.E.P. for the population as a whole (P), rather than the mean length of the period per individual (D). The original tables of DRIVER (1931) on *B*, and of LUCE (1931) on B^i have been obtained through the courtesy of Dr. A. H. HERSH. Inspection of DRIVER's data shows clearly that the factor of individual variability plays an important part in the duration of the T.E.P. as measured experimentally. At all temperatures used by DRIVER (except 22°), the coefficients of variability for facet number show definite, and in some cases, marked increases in those groups which were in the T.E.P. at the time of transfer. This increased variability, as pointed out earlier in this paper, is most easily accounted for by variability in the temporal position of the T.E.P. in individual flies of the population. The exception at 22° is especially interesting since it is consistent with the fact that at 22° the T.E.P., as determined by the writer from DRIVER's data, is relatively shorter than at other temperatures. It should be pointed out that the duration of the T.E.P. as established by the writer from these data is not fully in agreement with the conclusions of DRIVER, based upon the same data. There is, of course, a large subjective element entering into the determination of limits for the T.E.P. In general, the T.E.P.'s calculated by the writer from the data are longer than those presented in DRIVER's table 14. The T.E.P. curves for DRIVER's data show the typical sigmoid relation commonly found in data of this type.

LUCE's (1931) data on the T.E.P. in B^i differ in certain important respects from the data of either DRIVER or the writer, on *B*. For B^i, the beginning and end points of the T.E.P. can be selected with a high degree of precision. For the most part, LUCE's T.E.P. curves are not sigmoid in character and show quite definite intercepts with the control lines, rather than the asymptotic approaches which characterize the data on Bar. Correspondingly, only a very slight increase—in some cases no increase—in the coefficients of variability for facet counts is found in those groups transferred during the T.E.P. These results indicate that individual variability is not a very large source of error in LUCE's T.E.P. studies on B^i. The difference between *B* and B^i in individual variability in the T.E.P. is extremely interesting. This difference may be due to an inherent difference in the kinetics of facet determination in the two stocks. On the other hand, it may possibly be accounted for by greater uniformity, both genetic and environmental, in LUCE's experiments. Unfortunately, the data are inadequate for establishing the latter point.

The sigmoid character of the T.E.P. curves found in *B* has already

been emphasized. The fact that these curves are composite of both the time-facet function and the probability integral for individual variability in the temporal position of the T.E.P., has been demonstrated. We do not, of course, know the form of the time-facet function, but it is apparent that the superposition of the probability integral curves upon the time-facet curves is of itself adequate to account for the observed sigmoid relation. Moreover, if the interpretation of the kinetics of facet determination in *B*, presented earlier and reviewed here is correct, the time-facet curves will themselves be sigmoid.

GAUSE and ALPATOV (1932) have fitted logistic curves to the data of DRIVER (1926) on the T.E.P. in *B*. These writers have interpreted the curves as representing the time-course of facet determination. Although the logistic curve does give a fairly good fit for these data, it is at once apparent from the discussion presented earlier in this paper that the experimental curves obtained in the T.E.P. studies are composite curves and do not in any sense represent the real time-facet curve.

HERSH (1934) has recognized the compound nature of the T.E.P. curves, insofar as each point on the curve is composite of the number of facets determined at the beginning of the T.E.P. at one temperature and of the number of facets determined during the remainder of the T.E.P. at the second temperature. By a painstaking dissection of DRIVER'S 1931 data on facet counts in different transfer groups in *BB* (double-bar, or ultra-bar), HERSH has found that a symmetrical sigmoid curve representing the equation for an autocatalytic irreversible unimolecular reaction gives a satisfactory description of the time-course of the facet-determining reaction at each temperature studied. The observed points are in good agreement, within statistical limits, with the points calculated theoretically. HERSH points out that caution must be exercised in concluding from an analysis of this type that the calculated curve gives the true form of the time-facet function. He states, "Since consistent results are obtained a presumption is created that the assumptions made are to be accepted as being in agreement with the facts but one may not conclude that the resulting equations necessarily give the form of the true function. To draw such a conclusion it would need to be shown further that the form of function which gives consistent results is the only one that could possibly apply."

Apart from the exacting mathematical requirements which must be satisfied before any conclusions may be drawn relating to the form of the time-facet function, it is apparent from the considerations developed here that this type of analysis does not suffice to give a complete answer. To this end it is necessary to develop methods, either analytical or experimental, which make possible the resolution of the experimental T.E.P. curves into their component elements.

It was hoped that the general interpretation of the effect of *vg* on facet

number in *B* presented earlier might be tested quantitatively. For this purpose, exact information on the form of the time-facet function and the mean length of the T.E.P. in the individual is necessary. It has been demonstrated that these cannot be obtained from data of the type presented in this paper. Some contribution in this direction will be attempted by the application of new experimental methods, coupled with an extension of the types of experiments described here.

The results of the investigation on the effect of *vg* on facet number in *B* may be summarized as follows:

(1) The gene *vg* effects a small but significant diminution in facet number in Bar-eyed flies.

(2) Concurrently, *vg* effects a slight increase in the duration of the temperature-effective period for facet determination.

(3) The time limits of the temperature-effective period have been identified with the limits of a reaction which results in the decrease of the amount of material available for facet formation.

(4) The increase in the duration of the T.E.P. in *B vg* flies is adequate, qualitatively, to account for the decreased facet number, as compared with *B*.

(5) No quantitative test of the above interpretation has been possible due to limitations in the nature of the experimental methods which have been applied. The interpretation is, however, formally consistent with all the data available for the Bar series.

LITERATURE CITED

CHILD, G. P., 1935 Phenogenetic studies of scute-1 of *Drosophila melanogaster*. II. Genetics **20**: 127–155.

DRIVER, E., 1926 The temperature-effective period—the key to eye facet number in Drosophila. J. Exp. Zool. **46**: 317–332.

1931 Temperature and gene expression in Drosophila. J. Exp. Zool. **59**: 1–28.

GAUSE, G. F. and ALPATOV, W. W., 1932 Die logistische Kurve von Verhulst-Pearl und ihre Anwendung im Gebiet der quantitativen Biologie. Biol. Zbl. **51**: 1–14.

HERSH, A. H., 1934 The time curve of facet determination in an ultra-bar stock of *Drosophila melanogaster*. J. Gen. Physiol. **17**: 487–498.

KRAFKA, JOSEPH, JR., 1920 The effect of temperature upon facet number in the bar-eyed mutant of Drosophila. J. Gen. Physiol. **2**: 409–464.

LUCE, W. M., 1931 The temperature-effective period in infra-bar and its heterozygotes. J. Exp. Zool. **59**: 467–498.

LUDWIG, D. and CABLE, R. M., 1933 The effect of alternating temperatures on the pupal development of *Drosophila melanogaster* Meigen. Physiol. Zool. **6**: 493–508.

MARGOLIS, O. S., 1935 Studies on the Bar series of *Drosophila melanogaster*. I. Genetics **20**: 156–171.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. Proc. Sixth Int. Congress Genetics **1**: 213–255.

PLUNKETT, C. R., 1932 Temperature as a tool of research in phenogenetics: methods and results. Proc. Sixth Int. Congress Genetics **2**:158–160.

SEYSTER, E. W., 1919 Eye facet number as influenced by temperature in the bar-eyed mutant of *Drosophila melanogaster* (ampelophila). Biol. Bull. **37**: 168–181.

FURTHER STUDIES ON FACTOR INTERACTION IN DROSOPHILA VIRILIS

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INTRODUCTION

A character is a result of the cooperative action of all genes, although some genes alone, and in case of "factor interaction," more than one gene, are chiefly concerned in the development of a particular character. Interacting genes, as well as modifiers, may be specific in their effect, since they can act only in the presence of a particular genetical environment (BRIDGES 1919), and non-specific, when their action is not confined to a particular gene.

The present paper deals with the inheritance of thorax protrusions, curling of bristles and other characteristics which appear in various phenotypical expressions as a result of the interaction of certain genes with non-specific modifiers.

The following five genes are involved in the interaction here studied:

Ruffled (*ru*), a fifth chromosome recessive gene. In ruffled flies the tips of the dorso-central bristles and hairs near them are curled forward, and the thorax may be slightly telescoped (figure 1a).

Rounded (*R*), a second chromosome dominant gene. Rounded flies have truncate wings, short and bushy arista (figure 1d).

Clipped (*Cl*), a fourth chromosome dominant gene, lethal when homozygous. In clipped flies the posterior end of the wings are cut and the majority have two dorso-central bristles slightly curled (figure 1e).

Shaggy (*sh*), a fifth chromosome recessive gene. In shaggy flies the abdominal hairs are directed irregularly.

Beaded (*Bd*), a fifth chromosome dominant, lethal when homozygous. In beaded flies the wing margins are scalloped and one or two of the dorso-central bristles may become slightly curled, depending upon condition of the food and age of the flies.

The following are new characters which have resulted from the interaction between some of the above described genes:

Vortex-1, two well-developed horn-like protrusions on the thorax, located lateral to and midway between the anterior and posterior dorso-central bristles. The microchaetae or small hairs around these protrusions are arranged in a whorl.

Vortex-2, protrusions are only slightly developed, and in extreme cases leaving only two brown pigmented spots surrounded by whorled hairs.

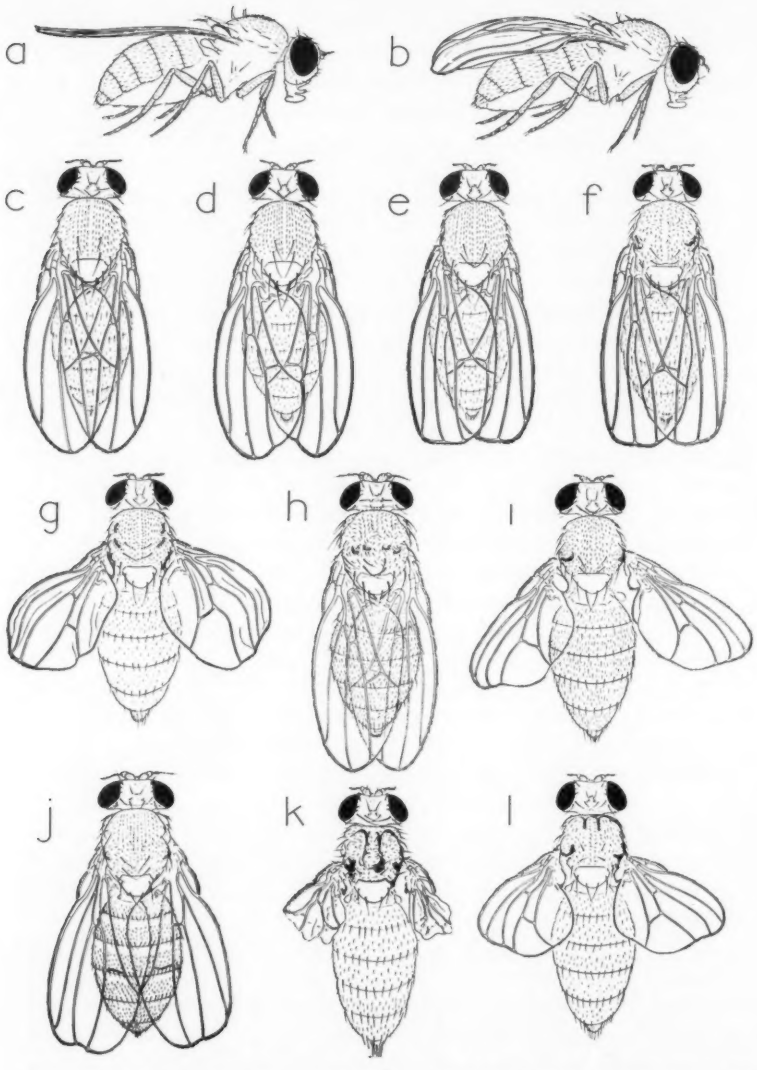

FIGURE 1.—(a) *ru/ru*, (b) *ru/+R/+*,(c) wild-type, (d) *R/R*, (e) *Cl/+*, (f) *ru/ru Cl/ +*, (g) *ru/ru R/R* and *ru/ru R/+*, (h) *ru/ru sh/sh* and *ru/ru sh/+*, (i) *R/R Cl/+* and *R/+Cl/+*, (j) *Cl/+ sh/sh* and *Cl/+sh/+*, (k) *ru/ru R/+Cl/+*, (l) *ru/+R/+Cl/+*. (Drawings by Miss E. DEG. MCKEE.)

Vortex-3, the pigmented spots lie in the wall of an indentation or funnel extending more or less deeply into the thorax. Very often two pairs of intrusions are present, which may be arranged either in pairs on the anterior and posterior parts of the thorax, or in a row across the thorax.

Curled bristles, tips of dorso-central bristles are curled forward, hairs near them being normal.

Extremely ruffled, dorso-central bristles and hairs near them are almost perpendicular to the thorax.

INTERACTION OF RUFFLED AND ROUNDED GENES

The ruffled (*ru*) gene becomes incompletely dominant with rounded (*R*). The change in dominance is accompanied by the appearance of a new characteristic called roofed in *ru*/+*R*/+ flies (figure 1b), and by an exaggeration of both rounded and ruffled characteristics as well as by the appearance of the new character vortex-3 in *ru*/*ru* *R*/*R* and *ru*/*ru* *R*/+ flies (figure 1g) (LEBEDEFF 1932).

Further studies (LEBEDEFF 1933) revealed that these characteristics, as well as others, also result from interaction of clipped with either ruffled or rounded.

INTERACTION OF RUFFLED AND CLIPPED

From thirteen crosses between ruffled (*ru*/*ru*) and clipped (*Cl*/+) flies, 375 wild-type and 428 clipped flies were obtained. The majority of the clipped flies from the cross had curled bristles and vortex-2 characteristic

TABLE 1

The variability of Cl/+ and Cl/+ru/+ flies.

	STRAIGHT BRISTLES (PERCENT)	CURLED BRISTLES (PERCENT)	CURLED BRISTLES VORTEX-2 (PERCENT)	NO. FLIES EXAMINED
Cl/+	11.75	77.45	10.8	315
Cl/+*ru*/+	1.2	33.7	65.1	428

of the thorax. The detailed studies of *Cl*/+ and *Cl*/+ *ru*/+ flies, as shown in table 1, show that the percent of flies having vortex-2 protrusions is 10.8 in *Cl*/+ flies and 65.1 in *Cl*/+ *ru*/+ flies. This indicates that the vortex-2 characteristic is a result of interaction between heterozygous ruffled and clipped genes.

From the backcross of F_2 *Cl*/+*ru*/+ flies to ruffled (*ru*/*ru*) four phenotypically distinct classes were obtained approximately equal in numbers (table 2a). There are two classes of clipped flies in the backcross population. One class (546 in number) is represented by flies having ruffled

bristles and vortex-2 characteristics. These flies resemble the F_1 clipped parent and evidently are genetically *Cl*/+*ru*/+. The other group (483) of clipped flies have the dorso-central bristles and adjacent hairs curled to a greater extent than the ruffled flies; the thorax of these flies has two very marked protrusions—vortex-1 (figure 1f). It is assumed that the vortex-1 character and the extreme ruffling of bristles and hairs is caused by the interaction of the homozygous ruffled and clipped genes, and that these flies are genetically *Cl*/+*ru*/*ru*. Wild-type (493) flies from the backcross have normal wings and straight bristles and hairs; genetically they are of +/+ *ru*/+ constitution. Ruffled (499) flies have the dorso-central bristles and adjacent hairs ruffled and have a somewhat telescoped thorax, and their genetical constitution is assumed to be +/+ *ru*/*ru*.

TABLE 2

Summary of backcross and F_2 data from Cl/+×ru/ru cross.

	NUMBER OF CULTURES	CLIPPED *Cl*/++/+	CLIPPED RUFFLED VORTEX-2 *Cl*/+*ru*/+	CLIPPED EXTREMELY RUFFLED VORTEX-1 *Cl*/+*ru*/*ru*	WILD-TYPE +/+ *ru*/+ OR +/++/+	RUFFLED +/+*ru*/*ru*
(a) *Cl*/+*ru*/*ru* ×*ru*/*ru*	26		546	483	493	499
Calculated			505	505	505	505
(b) *Cl*/+*ru*/+ ×*Cl*/+*ru*/+	16	203	394	217	295	95
Calculated		200.6	401.2	200.6	300.9	100.3

In the F_2 from the cross between clipped (*Cl*) and ruffled (*ru*/*ru*) flies, five classes appeared in a 2:4:2:3:1 ratio (table 2b). This ratio is a result of the interaction of the dominant clipped gene, which is lethal when homozygous, with recessive ruffled. The clipped curled vortex-2, clipped extremely ruffled vortex-1, wild-type and ruffled F_2 flies are the same as the corresponding classes in the backcross progeny. The additional clipped class of flies in F_2 are genetically *Cl*/+ +/+ constitution.

GENETICAL TEST OF F_2 PHENOTYPES

There are two classes in the F_2 progeny from the *Cl*/+×*ru*/*ru* cross, which phenotypically overlap each other: clipped and clipped ruffled vortex-2. As is shown in table 1, about 35 percent of *Cl*/+ *ru*/+ flies phenotypically cannot be distinguished from *Cl*/+ +/+ flies. Therefore the possibility of errors in classification of flies of these two classes cannot be excluded. In fact, out of the nine clipped ruffled vortex-2 F_2 flies which were tested, one proved to be clipped (*Cl*/+ +/+), since from the cross with ruffled (*ru*/*ru*) it gave 43 clipped ruffled vortex-2 and 39 wild-type flies. The other eight clipped ruffled vortex-2 flies gave 149 clipped ruffled

vortex-2, 135 clipped extremely ruffled vortex-1, 124 ruffled and 134 wild-type flies and thus proved to be *Cl*/+ *ru*/+.

Four clipped extremely ruffled vortex-1 F_2 flies, when crossed with ruffled flies, gave 134 clipped extremely ruffled vortex-1, and 156 ruffled flies, and thus proved to be of *Cl*/+ *ru*/*ru* constitution.

From three crosses between F_2 ruffled flies only ruffled were obtained, indicating that ruffled flies are double recessive +/+ *ru*/*ru*.

These genetical tests support the assumption that the ruffled vortex-2 characters are the result of an interaction of heterozygous clipped and heterozygous ruffled genes, and that the extreme ruffling of bristles and vortex-1 characters are the result of the interaction of heterozygous clipped and homozygous ruffled genes.

INTERACTION OF ROUNDED AND CLIPPED GENES

When it was found that ruffled interacts with rounded, as well as with clipped, it was of interest to determine whether rounded and clipped genes interact with each other. From eight crosses between rounded (*R*/*R*) and clipped (*Cl*/+) flies 281 rounded and 270 flies with rounded clipped extremely ruffled, vortex-1, and spread short roofed wings were obtained (figure 1i).

In order to test whether the ruffled gene was involved in this cross, since *R*/+ *Cl*/+ flies closely resemble *ru*/*ru* *R*/+, a cross was made between *R*/+ *Cl*/+ and wild-type flies. From this cross four distinct classes of equal numbers were obtained (182:177:171:189). The 182 rounded, clipped, extremely ruffled vortex-1, with spread, roofed short wing flies phenotypically resemble the *R*/+ *Cl*/+ parent and genetically are of the same constitution. The 177 rounded and the 171 clipped flies resemble the corresponding flies from stock cultures, and the 189 wild-type flies resemble their wild-type parent. This result shows that the ruffled gene was not involved in the cross, because if ruffled were present the rounded flies would be rounded, ruffled and roofed while the majority of clipped flies would have vortex-2, which was not the case. These data indicate that rounded and clipped genes interact to produce the characters vortex-1, extremely ruffled dorso-central bristles and adjacent hairs, and spread short roofed wings.

To find out what the phenotypical effect would be when *R*, *Cl* and *ru* genes are combined, F_1 flies from the cross *R*/+ *Cl*/+ × *ru*/*ru* were back-crossed to *ru*/*ru*. From the *R*/+ *Cl*+ × *ru*/*ru* cross three cultures were obtained, in which flies were of four classes approximately equal in number: (1) 64 flies were *R*/+ *Cl*/+ *ru*/+—rounded clipped vortex-1 extremely ruffled with spread short roofed wings (figure 11); (2) 60 were *R*/+ +/+ *ru*/+—rounded ruffled roofed; (3) 77 were +/+ *Cl*/+

ru/+—clipped vortex-2 ruffled and (4) 70 were +/+ +/+ *ru*/+—wild-type flies. The *R*/+ *Cl*+/ *ru*/+ flies differ phenotypically from *R*/+ *Cl*/+ flies only by having a deep furrow on the tip of the thorax. On the other hand, *R*/+ *Cl*/+ *ru*/*ru* flies, obtained together with other seven classes from *R*/+ *Cl*/+ *ru*/+×*ru*/*ru* cross, differ markedly from *R*/+ *Cl*/+ and *R*/+ *Cl*/+ *ru*/+ flies in having a deep furrow on the thorax extending almost half its length, resulting in a hump in the middle of the thorax; the wings of these flies are also greatly reduced, leaving only vestiges (figure 1k).

INTERACTION OF SHAGGY WITH RUFFLED AND CLIPPED

From nine crosses between shaggy (*sh*) and clipped (*Cl*) flies 396 clipped ruffled vortex-1 (figure 1j), and 389 wild-type flies were obtained. From seven cultures from the backcross of *Cl*/+ *sh*/+×*sh*/*sh* four classes appeared in approximately equal numbers: (1) 103 flies were *Cl*/+ *sh*/*sh*—clipped shaggy vortex-1 ruffled (figure 1j); (2) 97 were *Cl*/+ *sh*/+ clipped vortex-1 ruffled; (3) 101 were +/+ *sh*/*sh*-shaggy and (4) 111 were +/+ +/+—wild-type.

The *Cl*/+ *sh*/+ and *Cl*/+ *sh*/*sh* flies from this cross resemble each other as far as the ruffling of the dorso-central bristles and adjacent hairs, and vortex characteristics of the thorax are concerned. This indicates that clipped and shaggy interact with each other to produce vortex-1 and ruffled bristles and hairs on the thorax. The presence of *sh* in homozygous condition does not intensify this interaction.

A study of *ru*/*ru sh*/*sh* flies from a stock culture revealed that all have the vortex-3 character (figure 1h). From various crosses it was found that vortex-3 appeared also in *ru*/*ru sh*/+ flies, which indicates that vortex-3 appears also from interaction of homozygous ruffled with homo- or heterozygous shaggy.

INTERACTION OF BEADED WITH CLIPPED AND ROUNDED

Beaded (*Bd*) also can be added to this series of interacting genes. The interaction of this gene with the others is not conspicuous, since the bristle characteristics in beaded flies are affected by condition of food and by age. F_1 flies (*Bd*/+ *Cl*/+) from crosses between clipped and beaded were found to have curled bristles and vortex-2 thorax. But F_1 flies (*Bd*/+ *R*/+) from crosses between beaded and rounded were found to have curled bristles. No detailed statistical studies, however, were made either of *Bd*/+ *Cl*/+ or *Bd*/+ *R*/+ flies.

DISCUSSION AND SUMMARY

The interrelationship of five genes makes it possible to trace the role played by each gene in production of different characteristics. As it is

shown in the diagram (figure 2), the vortex-1 character appeared from the following combinations: *Cl/+ sh/sh, Cl/+ sh/+, Cl/+ ru/ru, Cl/+ R/R* and *Cl/+ R/+*, indicating that the clipped gene is primarily responsible for the vortex-1 character, but it does not appear unless in the presence of an intensifier, which can be either homozygous ruffled, or heterozygous shaggy or rounded genes. Similarly, vortex-2 also is due to the clipped gene, when intensified by heterozygous beaded or ruffled. However, vortex-3

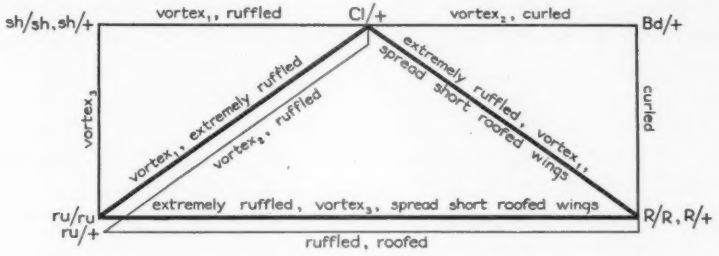

FIGURE 2.—New characteristics appearing in crosses involving five genes as the result of the interaction between these genes.

appears from *ru/ru sh/sh, ru/ru sh/+, ru/ru R/R* and *ru/ru R/+* combinations, indicating that the ruffled gene is primarily responsible for the vortex-3 character when it is intensified either by shaggy or rounded. Spread short roofed wings appeared in *R/R Cl/+, R/+ Cl/+, R/R ru/ru* and *R/+ ru/ru* flies suggesting that the rounded gene is responsible for this characteristic but appears only when rounded is modified by either clipped or ruffled. The extreme ruffling of bristles and hairs cannot be assigned to any particular gene, since it appeared in *Cl/+ ru/ru, ru/ru R/R, ru/ru R/+, Cl/+ R/R* and *Cl/+ R/+* flies, showing that any two of clipped, ruffled, and rounded genes can produce it.

These studies also revealed the important role played by genetic environment upon the phenotypical expression of the gene. In a particular environment the ruffled gene, for example, produces only curling of dorsocentral bristles and adjacent hairs, while under other conditions the same gene may produce various types of vortex protrusions on the thorax, exaggerated curling of bristles and hairs, and short spread and roofed wings.

LITERATURE CITED

BRIDGES, C. B., 1919 Specific modifiers of eosin eye color in *Drosophila melanogaster*. J. Exp. Zool. **28**: 337–384.

LEBEDEFF, G. A., 1932 Interaction of ruffled and rounded genes of *Drosophila virilis*. Proc. Nat. Acad. Sci. Washington **18**: 343–349.

1933 Studies on factor interaction in *Drosophila virilis*. Amer. Nat. **68**: 68–69.

THE PRODUCTION OF MUTATIONS IN DROSOPHILA MELANOGASTER BY IRRADIATION WITH ALPHA-RAYS

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INTRODUCTION

The artificial transmutation of the gene is an old problem. Investigators tried to produce mutations artificially but failed until 1927 when MULLER announced that gene mutations could be produced by means of X-rays. This suggested to his contemporaries the use of other agencies for this purpose. Since his announcement, other agencies that have experimentally been proved to produce a positive effect are beta and gamma radiation, and ultra-violet light. Moreover, increase in temperature has been shown to affect the mutation rate.

When I began working on this problem in 1932, no results had been published concerning the effects of alpha-radiation on Drosophila. Since then, HANSON published, in December of 1933, a note reporting that he had produced somatic variations and mosaics by means of alpha-radiation.

I thought it would be of interest to investigate the genetic effects of alpha-radiation, and particularly whether germinal mutations could be produced. An important property of alpha-radiation, which is not common to any of the other types of radiation tested above, is that the ionization is not uniform but is concentrated along the extremely narrow paths of the alpha-particles; and along these paths the ionization is extremely intense. On this account it seemed that some interesting results might be obtained with alpha-rays which do not occur with the other types of radiation.

The work was done under the direction and criticism of Dr. EDGAR ALTENBURG, for whose interest and suggestions I wish to express my sincere appreciation. I also wish to express my appreciation to Dr. L. M. MOTT-SMITH for his assistance in the calculations of this paper, in connection with the physical agent used. I wish to thank Mr. T. W. BONNER for furnishing the polonium source.

THE ALPHA-RADIATION

Polonium, radium F, was used as the source of alpha-radiation because it is the only pure source of alpha-radiation suitable for the purpose. The two sources used in the present experiments consisted of polonium plated on silver buttons, in one case seven millimeters in diameter, in the other, fourteen millimeters. The buttons were attached to the ends of glass rods, by means of which they were supported in suitable position for the irradiation of the material.

It may be well here to mention briefly some of the properties of the agency which are related to the problem. The alpha-radiation from polonium consists in the emission of high-energy particles. These particles are helium nuclei, having an atomic weight of four, and carrying a positive charge of two electronic charges. In the course of the radioactive disintegration these particles are ejected with considerable energy, and in consequence are able to produce various effects. Among these the two that have a direct bearing upon the explanation of the results obtained in these experiments are ionization of gases through which they pass, and the production of chemical changes of various kinds (such, for example, as the blackening of a photographic plate). The ability to ionize is perhaps fundamental to all chemical effects. It has been found that in all reactions that take place under the influence of alpha-ray bombardment, the rate of reaction is proportional to the amount of ionization. This ionization can be calculated if the strength of the source, the duration of the treatment and the distance from the source to the irradiated material are known. Another quantity which is of importance is the total number of ions produced in air by each alpha-particle. In the case of polonium the initial energy of the alpha-particle is 5.2×10^6 electron volts. Since in air it is known that about thirty volts of energy are required to produce one pair of ions, the division of this quantity into the total energy of one alpha-particle gives the total number of ions which an alpha-particle will form, namely, 170,000 ions. It is of interest to note that this ionization is produced in a very small volume. In the case of a polonium alpha-particle ejected from a thin film of polonium into the surrounding air, it is known that the particle proceeds away from the source along a rectilinear path and is finally stopped after traveling a distance of 3.9 cm in air under standard conditions of temperature and pressure (76 cm, 15 C). The distance is known as the range of the alpha-particle and is the same for all particles of a given element. The 170,000 ions are all formed, excepting a very occasional delta-ray, within a narrow cylinder, whose axis coincides with the path of the ray. Its length is, of course, 3.9 cm, while its diameter is probably not more than one hundred atomic diameters. In the case of an alpha-particle traversing a solid or a liquid material, the effects are the same, except that the range is greatly reduced on account of the much greater density of the material. The range in any material can be calculated if the atomic constitution and the density of the material are known. In the case of the material of the egg (assumed to contain carbon, hydrogen and oxygen in the proportion of starch and of density one gram per cubic centimeter) the range was calculated to be 50 microns. On the other hand, the total number of ions formed must be of the same order of magnitude as the number found for air. At least it is certain that 5.2 million volts

energy will be used up in producing all sorts of disruptions of the relatively easily disarranged organic molecules composing the egg material. Since the average binding power of the average organic bond is probably not more than two volts, perhaps two million molecules will be decomposed along the narrow cylindrical path of one single alpha-particle, which has now shrunk to a length of only 50 microns. Accordingly, if an alpha-particle enters a cell, it must cause considerable chemical or ionizing effect.

Since the maximum distance which the radiation can travel away from the source is only 3.9 cm, and also, since the alpha-particle is losing energy along its path, it is very important to treat the material at a small and constant distance from the source. In all the experiments to be described this distance was maintained at between four and five millimeters.

THE METHODS EMPLOYED TO OVERCOME THE POOR PENETRATING POWER OF THE ALPHA-RAYS

There were many difficulties to be overcome in the use of alpha-irradiation. The first difficulty was obtaining a polonium source that covered a sufficient area for irradiating a large number of eggs. The source used in this experiment was described in the preceding section on alpha-radiation. On account of the poor penetrating power of this agent it was impossible to employ the usual method of raying adult flies. For a substance having the density of fly material, the calculated penetrating power is approximately 50 microns. It is, therefore, impossible to produce germinal mutations by alpha-irradiation of the adults. This necessitated the use of the eggs. However, the penetrating power is so small that it is necessary to irradiate the eggs when the reproductive cells are in a peripheral position. This procedure was adopted by GEIGY in his work with ultra-violet light. Since this peripheral position of the germ cells persists for not more than one hour during the development of the egg at 22°C or above, it was necessary to collect eggs of the same age in large quantities.

Two methods similar to the method used by GEIGY in his work with ultra-violet light, for obtaining the eggs were employed. In the earlier experiments the flies were collected while young, and the females separated from the males. The flies were kept on separate fresh food for three to five days, and were then mated. The flies copulated within ten to fifteen minutes after they were placed together, and the females began depositing eggs within from two to three hours. After the females began depositing eggs in large quantities, they were transferred to a second bottle of fresh food which contained a few drops of three percent acetic acid on a piece of filter paper. The eggs in the first bottle were rejected because microscopic examination showed that the eggs were in different stages of development. The females were then allowed to lay on the food of a second

bottle for a period of from one hour to one hour and ten minutes. Since the first batch of eggs was discarded, it was not necessary to keep the flies as virgins, but merely to collect them while young and allow them to be well fertilized. They were kept on food well worked with larvae from three to five days old because the females would not deposit their eggs until they were placed on fresh food. If the surface of the food was very moist, the females did not partially bury their eggs and this facilitated the removal of the eggs from the food. It was possible by the above method to collect from five hundred to one thousand eggs from approximately two hundred females in the one-hour period. Incidentally, if the flies had been subjected to a lowering of the temperature several degrees the day before the experiment, the number of eggs laid was much less, at times even insufficient for the experiment.

The irradiation in this definite stage is facilitated by an examination of a sample of the living eggs. The time factor by itself cannot be used since the stage of the egg is dependent upon the rate of development and the age of the egg when collected. It is impossible to determine the exact time of fertilization of all of the eggs because, first, the females may retain the egg in the oviduct after fertilization, and thus lay an occasional late egg; second, the rate of development is affected by the temperature of the egg. The examination was made by the method described by CHILD and HOWLAND in 1933 in a report on "Observations on Early Developmental Processes in the Living Egg of Drosophila."

The eggs to be examined were kept under conditions identical with those of the eggs to be treated. Those for examination were removed about ten minutes before the calculated time for raying. A smear was made with yeast-fermented food on a clean slide and the eggs placed on it. The smear was allowed to dry for two or three minutes, after which the eggs were easily dechorionated by the use of a sharp needle. The eggs were then transferred to a drop of water on a clean slide. One piece of 0.2 mm cover glass was used on either side of the drop to protect the eggs from the weight of the cover slip. A 0.1 mm cover glass was placed over the drop, and the eggs were examined under the microscope. The eggs were usually in the same stage of development. In an occasional experiment one out of the ten eggs was too late, that is, the germ cells had migrated into a depression on the dorsal surface. The age of the eggs at the time of examination was from two to two and one-half hours. The time during which the polar cells were in a terminal position was approximately one hour, at 22° C, after which they migrated rapidly into the invagination on the dorsal surface. When the eggs were examined from two to two and one-half hours after they were collected, "early polar caps" had made their appearance. The germ cells were usually large and few in number, from four to

six in the side view. They were not delimited from the remainder of the egg material. Approximately ten minutes later they were in the "median polar-cap" stage. At this stage there were in the polar-cap approximately ten primordial germ cells in the side view of the egg, and these were delimited from the remainder of the egg material. The somatic cells had developed and were one cell layer in thickness around the periphery of the egg. Approximately ten minutes later the eggs were in the "late polar-cap" stage. The pole cells were more numerous and the somatic cells were two cell layers in thickness around the periphery of the egg (see figures 1 and 2). The total time interval from the first appearance of the germ cells to their migration is approximately one hour.

Photo-micrographs of living Drosophila eggs

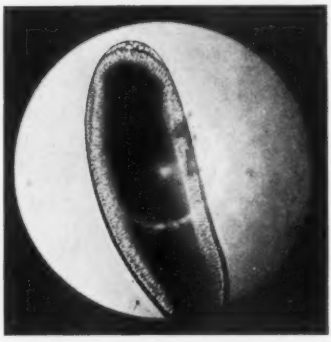

FIGURE 1.—Lateral view of egg in the "late" polar-cap stage. Magnification 160×.

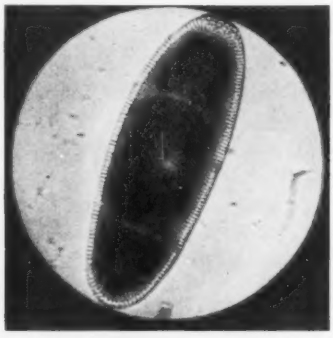

FIGURE 2.—Ventral view of egg in the "late" polar-cap stage. Magnification 160×.

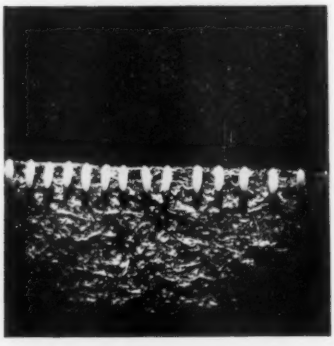

FIGURE 3.—Eggs aligned for raying viewed from above. Magnification 15×.

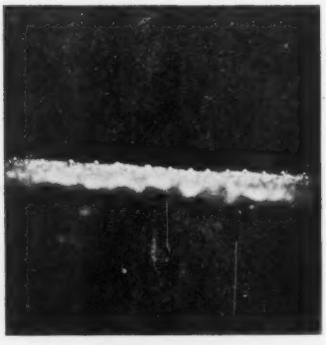

FIGURE 4.—Eggs aligned for raying as seen by the source. Magnification 15×.

The eggs for the different experiments were rayed when they were in either the "median" or "late" polar-cap stage, and were examined just before and during the time of irradiation.

The irradiation of the eggs introduced the problem of somatic effects. If the eggs were irradiated during this early stage, approximately two and one-half hours after segmentation had begun, a dosage that produced a germinal mutation should also produce a somatic mutation, since all of the somatic cells are likewise in the peripheral position at this stage of development. Since at this stage the number of somatic cells is very small, only two layers around the periphery, any somatic mutations will be manifested in a large area of the fly, and might prevent it from developing to maturity. Or, if a large number of the cells were killed, the fly again could not develop. This was found to be true in several early experiments, as will be presented in subsequent paragraphs. This difficulty was avoided in the final experiments by shielding from the radiation approximately ninety-five percent of the egg (figures 3 and 4) as suggested by GEIGY's work. This was possible since the reproductive cells are in a terminal position as described above. Since the eggs are flat on the dorsal side, the amount shielded could be determined accurately.

EXPERIMENTS ON THE PRODUCTION OF TRANSLOCATIONS

The effects of alpha-rays in producing translocations was first tried because the occurrence of translocations under natural conditions is extremely rare, while numerous translocations have been found with X-ray treatment. This circumstance eliminates the necessity of control experiments and the testing of a large number of the germ cells.

The eggs for these experiments were obtained by the method previously described and were arranged for treatment in the following manner. A small dish was filled with hot, liquid banana-karo-agar food. On cooling it solidified with a smooth flat surface. The eggs were immersed in the food vertically with their amicropolar ends upward and the micropolar end about two-thirds covered with food. They were arranged in rows, usually five, containing eight eggs each, and covering an area somewhat smaller than that of the seven millimeter diameter source-button. They were treated by being placed directly under the source and at a distance of four to five millimeters from it. Preliminary tests showed that treatment for forty seconds or more with the strength of source in use at this time (0.10 millicurie) damaged the cells to such an extent that adult flies could not develop. By varying the time of treatment it was determined that ten to twenty seconds gave the most suitable dosage.

In these experiments the age of the eggs was from two to two and one-half hours. During this interval they were in the "median" polar-cap stage of development.

Tests were made to detect translocations between the second, third and fourth chromosomes of wild stock eggs. The males which hatched from the eggs were mated to virgin females homozygous for recessive traits in these chromosomes. These traits are brown eye color, ebony body color and eyeless, respectively. The sons from the above cross were backcrossed to brown, ebony, eyeless virgins. The F_1 cultures of F_2 offspring were then examined for the presence of the three phenotypic classes, brown, ebony and eyeless.

The results obtained from these experiments are shown in table 1.

TABLE 1

Translocation experiments.

EXPERIMENT NUMBER	TREATMENT SECONDS	NO. EGGS	NO. FEMALES	NO. MALES	NO. MALES MATED	NO. MALES STERILE	NO. MALES TESTED	F_1 CULTURES	TRANSLOCATIONS	SOMATIC MUTATIONS
2.0	20	80	11	12	12	4	8	508	0	0
2.1	10	30	4	9	9	3	6	256	0	3
2.2	24	30	7	12	5	2	3	256	0	0
3.0	20	90	3	5	4	1	3	159	0	0
4.1	24	56	5	7	6	1	5	374	0	3
4.2	20	162	11	11	9	4	5	459	0	1
Totals		448	41	56	45	15	30	2012	0	7

From the above table it will be seen that from the 448 treated eggs, ninety-seven flies developed to maturity. Of these fifty-six were males and forty-one females. Forty-five of the males were mated in individual cultures to brown, ebony, eyeless virgins. Thirty of the forty-five males were fertile. From the fertile males 2012 F_1 cultures were examined for translocations. No translocations were observed. Seven and two-tenths percent (7.2 percent) of the ninety-seven flies which developed to maturity showed somatic variations. Out of the forty-five males which were tested fifteen (or 33 percent) were sterile. The part of this sterility caused by the alpha-rays is obtained by subtracting from this figure the percent of sterility which occurs naturally. No controls were carried through with these experiments, but the percentage of natural sterility can be obtained approximately from the controls of the other experiments of this paper. This procedure is legitimate because the controls for the other experiments were run under similar conditions of environment, particularly with regard to the temperature. This figure was found to be three percent so that the corrected sterility was thirty percent.

The fact that no translocations were observed may have been due to insufficient shielding of the somatic cells, which prevented the use of a sufficiently large dose.

PRELIMINARY EXPERIMENTS ON THE PRODUCTION OF LETHAL MUTATIONS

The eggs for these experiments were collected and a sample examined by the method described in the second section of this paper. The eggs were arranged for treatment in the same manner as the experiments on translocations. It was found that with the 0.17 millicurie source in use at this time the lethal dose was about thirty seconds, and that ten seconds was the most suitable dose.

MULLER'S well-known *ClB* method was used to detect the occurrence of lethal mutations in the X chromosomes. The genes for vermilion eyes and forked bristles were used in the other X chromosomes of the *ClB* stock in order that linkage tests could be made by testing the percent of crossing over between eosin and vermilion, and between vermilion and forked in order to locate the lethals as to one of these regions.

The lethals were divided into two types: "natural" lethals, which would occur at any time during the life of the fly, and those that occurred during the "polar-cap" stage (ALTENBURG 1933). Those occurring in the polar-cap stage can be distinguished from the ordinary lethals because at this stage the number of reproductive cells is smaller (between 2 and 30). Thus, at least three percent of the offspring should carry the lethal, and in addition linkage tests should show that the lethals are all in the same region of the X chromosome. Linkage tests were made to confirm the supposition that they were from the same parent cell. Lethals of this type were termed "re-duplicated" lethals. If less than four percent of the offspring carried the lethal it was classified with the "natural" lethals.

It is evident that the occurrence of "re-duplicated" lethals in the treated eggs must be due, definitely, to an effect of the treatment because the probability that a "re-duplicated" lethal should occur by chance is extremely small. This probability may be calculated as follows:

If a lethal occurs, the probability of its occurrence during any one hour is 1/400 since the total life over which the fly was tested was, on the average, seventeen days or about 400 hours. The average "natural" mutation rate of all of the controls of these experiments was 1/600, which means that there is one chance in 600 that one cell will mutate in the 400 hours. Assume that a mutation is equally likely to occur at any time during any stage of the life of the fly. The chance that one cell will mutate in one hour is 1/600 times 1/400, or 1/240,000. Now since the eggs at this stage have not more than an average of thirty primordial germ cells, the chance that one of these thirty cells will mutate is 30/600 times 1/400, or 1/8000. Therefore, the chance that one female will contain a "re-duplicated" lethal is 1/8000.

In looking for "re-duplicated" lethals it was not necessary to test more than from fifty to sixty F_1 cultures per treated male, since at least three percent of the offspring are expected to show the lethal. For instance, according to the calculations of Dr. HEWITT DIX, the chances are only one in sixteen of overlooking a lethal that is re-duplicated in 5 percent of the F_1 offspring, if 54 such offspring are bred. When a lethal was found, if possible an additional fifty F_1 cultures from this male were made to determine more accurately the percent of F_1 offspring that contained the lethal.

The results are presented in table 2. The amount of dosage given in the table is in millicurie-seconds, calculated by multiplying the effective strength of the source at the time of treatment by the duration of the treatment in seconds.

TABLE 2

Preliminary lethal experiments.

	TREATMENT MILLICURIE SECONDS	NO. EGGS	NO. FEMALES	NO. MALES	NO. MALES TESTED	NO. MALES STERILE	NO. MALES DEAD	NO. MALES TESTED	NO. F_1 CULTURE COUNTS	"NATURAL" LETHAL	"RE-DUPLICATED" LETHAL
Treated	1.4–2.4	966	80	63	42	8	4	36	1983	4	0
Controls	0	384	65	57	36	2	1	34	2135	5	0

From the above table it will be seen that a total of 966 eggs were rayed. One hundred forty-three, or 15 percent, developed to maturity. Of these 63 were males. Out of 42 males mated to *ClB* virgins four died and eight were sterile. From the 30 fertile males 1983 F_1 cultures were examined. From these F_1 cultures four "natural" lethals were obtained and located in region I or II. No male tested carried a "re-duplicated" lethal.

A total of 384 eggs were handled in the same manner as the above series except that they were not exposed to the alpha-radiation. From the 384 eggs, 122, or 30 percent developed to maturity. Of these 57 were males. Out of 36 males mated one died and two were sterile. From the 34 fertile males 2135 F_1 cultures were made. From these F_1 cultures five "natural" lethal mutations were obtained and located in region I (between *we* and *v*) or II (between *v* and *f*).

Twenty-one percent sterility of the treated, minus 5.5 percent sterility of the controls is equal to 15.5 percent, the approximate percentage of sterility due to the treatment.

A significant difference in the results obtained from the treated and control eggs was that 50 percent as many eggs developed to maturity in the treated as in the controls. It is evident from the number of eggs killed by the treatment that the somatic cells should be more adequately shielded.

The number of males tested was not sufficient to prove whether lethal mutations could be produced with the strength of the dose used.

THE PRODUCTION OF LETHAL MUTATIONS

The somatic cells of this group of experiments were more adequately shielded. The eggs were placed in a line as shown in figure 3. The dorsal surface of the egg was against the shield with the micro-polar tip of the egg projecting over the edge. The shield was a piece of blotting paper the edge of which was cut at an angle. The blotting paper was kept moist because the water prevented desiccation and its surface tension held the eggs in position. The eggs extended in a line 12 mm in length, with from twenty to thirty eggs per line. The length of the line was limited because the alpha-ray source had a radiating surface of only 14 mm in diameter. Two lines of eggs were irradiated at a single raying. The blotting paper was placed on a slide with the edge upon which the eggs were aligned projecting. This facilitated the handling of the eggs.

All of the eggs were placed with approximately five percent of the egg projecting (figure 4). Thus, practically all of the somatic cells were shielded. This allowed a larger dosage to be given. The eggs were rayed in the "median" and "late" polar-cap stages, which were described previously.

The *ClB* method was used to detect lethals and the F_1 cultures were examined for "natural" and "re-duplicated" lethals, as in the preliminary experiments.

These experiments are divided into two groups, those in which the eggs were dechorionated and those in which they were not.

Experiments with dechorionated eggs

It was thought that better results might be obtained if the chorion was removed from the eggs, as this procedure would allow the alpha-particles to penetrate further into the egg.

The eggs used as controls were treated in an identical manner as the rayed eggs except that they were not given the treatment.

The experimental results are shown in table 3.

TABLE 3

Lethal experiments using dechorionated eggs.

	TREATMENT MILLICURIE SECONDS	NO. EGGS	NO. FEMALES	NO. MALES	NO. MALES MATED	NO. MALES STERILE	NO. MALES DEAD	NO. MALES TESTED	NO. F_1 CULTURE COUNTS	"NATURAL" LETHALS	"RE-DUPLICATED" LETHALS
Treated	0.3–2.5	486	106	121	103	5s	6d	92	4520	10	1
Control	0	262	52	55	48	2s	1d	45	1523	6	0

From a total of 486 eggs treated 227 adults were obtained. Of the 121 males that developed, 103 were mated. Of the males tested five were sterile and six were dead six days after they were mated. From the 92 fertile males one "re-duplicated" lethal was obtained and one sex-linked, "re-duplicated" visible wing mutation. The number of F_1 cultures from the 92 fertile males was 4520, from which ten "natural" lethals were obtained and located as to region I or II (table 4).

From 262 control eggs 107 flies developed. Out of the 107 flies that developed 55 were males. Out of the 48 males that were mated two were sterile and one dead. From the 45 fertile males, 1523 F_1 cultures were examined and six "natural" lethals obtained and located as to region I or II (table 4).

TABLE 4

"Natural" lethals from dechorionated eggs.

TREATED					CONTROLS				
EXPERIMENT NUMBER	NUMBER F_1: WITH LETHAL	NUMBER F_1 TESTED	I (*vc-v*)	II(*v-f*)	EXPERIMENT NUMBER	NUMBER F_1 : WITH LETHAL	NUMBER F_1 TESTED	I(*vc-v*)	II(*v-f*)
L12.1	1 :	80		II					
L12.2	1 :	118	I[a]	(semi-					
L12.2	1 :	27	I	lethal)					
L13.2	2 :	121		II					
L18.1	1 :	52		II	CL18	1 :	99		II
L18.1	1 :	106		I	CL18	1 :	76	I	
					CL18	1 :	78	I	
L20	1 :	63		I	CL20	1 :	21		II
L20	2 :	61	I	II					
L21	1 :	50	lost		CL21	1 :	53		II
					CL21	1 :	69	I	

Experiments with undechorionated eggs

The lethal dose was not determined since the somatic cells were very adequately shielded. The dose was varied from 1.6 to 10 millicurie seconds. The adequate protection of the somatic cells permitted the use of a dosage four times as great as the dose of the preliminary experiments.

The results are shown in table 5. A total of 1135 eggs were treated. Of these 536 developed to maturity. Out of the total 272 were males. The number of males mated was 254. Of the 254 mated 80 were sterile and 15 dead. Of the 149 fertile males some were semi-fertile and an average of only forty-two and two-tenths (42.2) F_1 cultures could be tested per male. In the 7366 F_1 cultures twenty-two "natural" lethals occurred (table 6).

TABLE 5

Lethal experiments using undechorionated eggs.

EXPERIMENT NUMBER	TREATMENT MILLI-CURIE-SECONDS	NO. EGGS	NO. FEMALES	NO. MALES	NO. MALES MATED	NO. MALES STERILE	NO. MALES DEAD	NO. MALES TESTED	NO. F_1 CULTURE COUNTS	"NATURAL" LETHALS	"RE-DUPLICATED" LETHALS	AVERAGE NO. F_1 CULTURES PER MALE TESTED
L14.1	1.6	18	3	10	10	1s		9	462	0	0	51
L14.2	3.1	13	3	7	7		1d	6	373	1	0	54
L14.3	4.8	31	5	3	3		2d	1	122	0	0	54
L14.4	6.4	20	3	1	1		1d	0	0	0	0	0
CL14	0.0	23	12	9	9	1s		8	441	1	0	51
L15.1	6.3	44	12	8	8	0		8	349	0	0	44
L15.2	9.5	26	9	4	4	2s	1d	1	95	0	1	49
L15.3	12.6	25	0	3	2	1s	1d	1	40	0	0	40
L15.4	18.9	21	1	0	0	0		0	0	0	0	0
L15.5	15.2	19	2	0	0	0		0	0	0	0	0
CL15	0.0	17	6	6	6	0		6	323	0	0	54
L16	3.8	72	31	23	22	3s		18	694	3	0	35
CL16	0.0	50	7	13	13	0		13	699	2	0	51
L17	3.8	142	45	45	40	10s		30	1219	5	0	39
CL17.1	0.0	72	17	29	28	1s		25	1063	1	0	43
CL17.2	0.0	44	14	15	8	0		8	368	0	0	46
L19	5.5	84	21	26	26	4s	1d	20	965	3	3	45
CL19	0.0	81	27	30	30	0		25	1276	5	0	47
L22.1	7.7	43	4	5	5	4s		1	83	0	1	52
L22.2	10.0	50	3	7	6	5s		1	42	0	0	42
CL22	0.0	60	11	6	6	0		3	163	0	0	53
L23	8.6	99	26	37	34	25s	1d	8	482	2	0	52
CL23	0.0	84	33	36	36	1s		26	1473	1	0	54
L24.1	8.6	74	29	26	23	7s		16	911	3	1	51
L24.2	8.6	30	9	11	10	7s		3	129	0	0	43
CL24.1	0.0	77	30	32	26	2s	1d	23	1235	0	0	53
CL24.2	0.0	78	36	28	8	0		8	409	0	0	51
L25	7.9	116	23	27	26	11s		15	898	2	1	44
CL25	0.0	110	30	24	24	0		20	1073	1	0	49
L26	5.8	110	11	12	7	4s		3	134	1	0	32
CL26	0.0	96	17	17	2	0		2	112	0	0	56
L27	7.0	98	24	17	13	5s		8	368	1	0	40
CL27	0.0	118	8	11	11	1s		10	524	0	0	52
Treated Totals		1135	264	272	254	80s	15d	149	7366	22	7	42.2
Control Totals		910	248	227	210	6s	4d	177	9159	11	0	51.8

Of the 149 males tested seven (or 4.2 percent) contained "re-duplicated" lethals (table 7).

TABLE 6

"Natural" lethals from undechorionated eggs.

TREATED					CONTROLS			
EXPERIMENT NUMBER	NO. F_1 WITH LETHAL	:	NO. F_1 TESTED	I(ivc-v) v-NEAR VERMILION II(v-f)	EXPERIMENT NUMBER	NO. F_1 WITH LETHAL	: NO. F_1 TESTED	I(ivc-v) II(v-f)
L14.1	1	:	53	II[a]	CL14	1	: 91	I
L14.2	1	:	102	II[a]				
L16	1	:	34	I	CL16	1	: 88	II
L16	1	:	32	I	CL16	1	: 54	II
L16	4	:	107	4II[a]				
L17	1	:	78	II	CL17	1	: 35	v
L17	1	:	54	I				
L17	1	:	26	II[a]				
L17	1	:	13	I				
L17	1	:	12	II				
L19	1	:	69	I	CL19	2	: 84	I, II
L19	1	:	54	I	CL19	1	: 69	I
L19	1	:	36	lost	CL19	1	: 54	I
L23	2	:	119	2II	CL23	1	: 88	II
L23	2	:	98	2II				
L24	1	:	85	II				
L24	1	:	84	I				
L24	1	:	55	I				
L25	1	:	93	I	CL25	1	: 53	II
L25	1	:	57	I				
L26	1	:	20	I				
L27	3	:	110	2I, 1 lost				

From a total of 910 control eggs 475 developed to maturity. Of these 227 were males. Out of the 210 males mated, six were sterile and four dead. Because of the large number of sterile males in the treated, F_1 culture counts were made for only 177 of the 200 fertile males. From these 177 fertile males an average of fifty-one and eight-tenths (51.8) F_1 culture counts per male were made. In a total of 9,159 F_1 cultures eleven "natural" lethals occurred (table 6). No "re-duplicated" lethals were found.

The probability that seven "re-duplicated" lethals should occur by chance is extremely small and is calculated as follows. The calculations in the section on preliminary experiments show that the probability of a mutation occurring in one egg "naturally" during the one hour at the time

of raying is one in 8000 when the natural rate is one in 600. The chance that one of the 149 eggs which were treated will mutate is 149/8000, or 1/54. The probability that seven cells undergo a mutation is extremely small, and was calculated to be $1/7.5\times10^{15}$.

It is of interest to calculate the amount by which the treatment increases the mutation rate. To do this one first calculates the chance that a mutation will occur "naturally" during the interval of time that the eggs were being irradiated, and then compares this rate with the rate found for the treated eggs. As shown above, the chance that a natural mutation will occur in 149 eggs during one hour is 1/54, but the treatment lasted approximately five minutes in this series of experiments. Consequently, the chance that a natural mutation will occur in five minutes is $1/54\times5/60$, or 1/648. Therefore, one such mutation will occur on the average in 648×149, or 97,000 eggs. Actually seven "re-duplicated" mutations occurred in the 149 eggs, or one in about 21 eggs. Thus, the treatment increased the mutation rate by the factor 97,000/21, or 4600 times.

The natural mutation rate of the controls of this series of experiments was unusually low, 1/833. Therefore, the natural mutation rate of the controls of all of the experiments on shielded eggs was used in the calculations. This is legitimate because the series on undechorionated and those on dechorionated eggs were run intermittently. The "natural" mutation rate of the treated flies in this series of experiments was unusually high, 1/335. It is ascribed to two factors: first, a part of the difference can be accounted for by the fact that several cases of lethals which were probably really "re-duplicated" were classified as "natural" because less than four percent of the offspring contained the lethal, so that they could not positively be classified with the mutations which occurred within the one hour at the time of raying. Actually, four such cases were obtained; second, a part of the difference can be ascribed to failure of obtaining a sufficient number of tests per male, because of the semi-sterility of the males. Three such cases of lethals were obtained in which only 20 reproductive cells, or less, were tested per male. No such cases of either type were found in the controls (table 6). The conclusion that alpha-rays have an effect is based on the seven positive cases of "re-duplicated" lethals, as shown in table 7.

The high percent of offspring which contain the lethal in all cases shown in table 7 is probably due to the large number of the primordial cells that were killed by the irradiation. If a lethal occurred in the "median" to "late" polar-cap stage and no cells were killed, approximately three percent of the F_1 offspring would be expected to contain the lethal. In two cases 100 percent of the offspring carried the lethal. This result may be interpreted in two ways: It may mean that the egg which gave this large percentage of lethals was in a very early polar-cap stage when there were

TABLE 7

"Re-duplicated" lethals.

EXPERIMENT NUMBER	DOSAGE MILLICURIE-SECONDS	NO. F_1 CULTURES	NO. F_1 CULTURES CONTAINING LETHAL	PERCENT F_1 CULTURES CONT. LETHAL	LOCATION OF LETHAL IN REG. I OR II
L15.2	9.5	89	89	100	I
L19	5.5	80	14	19.3	II
L19	5.5	24	2	8.3	II
L19	5.5	19	2	10.5	I
L22	7.7	83	16	19.3	II
L24	8.6	54	54	100	I
L25	7.9	49	7	14.3	I

only a few cells, and that all but one of these cells happened to be killed, and the remaining cell contained the lethal. On the other hand, since none of the eggs examined as samples in any of these experiments were found to be in such an early stage, it is more likely that the result was simply due to the killing of all of the cells but one in an egg in a late stage. The percentage of cells which were killed by the treatment was found to be very high as is shown in the next section.

STERILIZATION PRODUCED BY ALPHA-RAYS

Beginning with experiment L16, a careful check was made of the percentage of sterility occurring in the males. In these experiments the age of the eggs at the time of raying was between two hours and ten minutes and three hours and ten minutes, and the males were mated on an average of twelve days later. They were examined for sterility during the next twelve days. As a precaution, a second brood was made six days after mating, because it was possible that if the males regained fertility sixteen or seventeen days after treatment, fertile eggs laid on old food might not develop. Control males were tested in the same manner.

In making a comparison of the amount of sterility with the alpha-ray dosage, only the experiments on the unshelled eggs from L16 to L27 were taken. This was done because the sterility was not only more carefully tested here than in the earlier experiments, but also because between this and later series and the earlier ones certain changes were made in the arrangement of the source. These changes altered the effective strength of the source so that it was not possible to compare accurately the dosage of the two series.

The collected results are shown in table 8. Only those males which were found to be permanently sterile were included in the sterile group. Some males were found which were semi-sterile to the extent that they produced only fifteen to twenty offspring. These were classed as fertile. It is seen that a large percentage of sterility was produced and that it increases

TABLE 8

The relation between the percentage of sterility and the dosage.

DOSE	NO. MALES	NO. STERILE	PERCENT STERILITY
0.0	183	6	3.3± 0.97
3.8	60	12	20 ± 3.9
5.6	39	8	26 ± 4.9
7.6	44	20	45 ± 6.8
8.6	66	39	59 ± 6.4
10.0	6	5	83 ±25.0

rapidly with the dosage. Its relation to the amount of treatment is also presented graphically in figure 5.

The amount of sterility found in these experiments may be compared with that found by MOORE for eggs irradiated with X-rays. This comparison is made in table 9. In order to render comparable the dosages in the

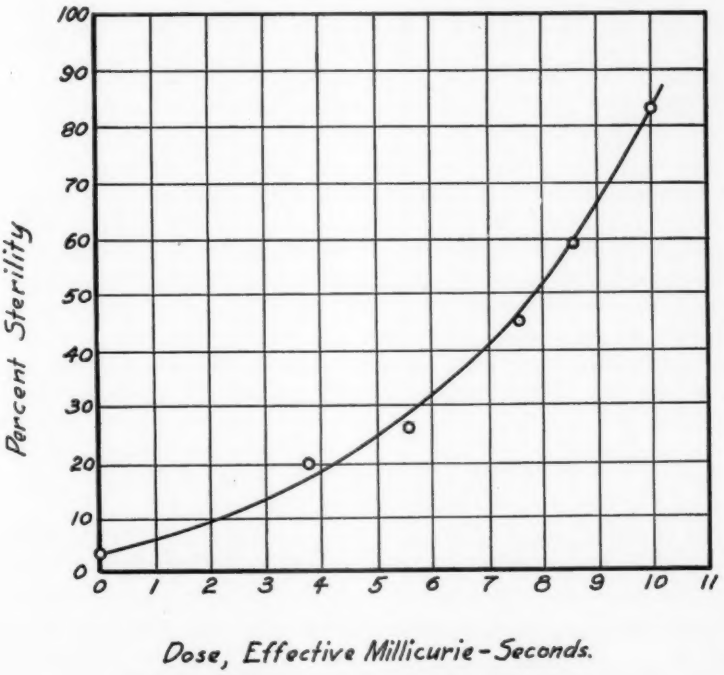

FIGURE 5.—Sterility-dosage curve. Experiments L16–L27.

two cases, they have been converted to ionization (ionization as a measure of the dosage is discussed in a later section). The data show that the alpha-radiation seems to give a significantly greater percentage of sterility. The

alpha-ray dose which has been chosen is the highest for which reliable experimental figures were obtained, but it is lower than that used by MOORE with X-rays. On the other hand, the alpha-ray sterility is higher than the X-ray, and, judging from the course of the curve, would be considerably higher with an alpha-ray dose equal to the X-ray dose. This result is probably due to the concentrated character of the alpha-ray ionization, which makes alpha-rays more effective than X-rays in damaging the cells. However, the eggs treated were at different stages of development, and this could be interpreted as the reason for the difference in effect of the two agents.

TABLE 9

	DOSAGE	IONIZATION	HOURS AGE	NO. MALES	NO. STERILE	PERCENT STER. OBS.	PERCENT STER. OF CONTROL	PERCENT STER. DUE TO TREATMENT
MOORE	1325 r units	2.8×10^{12}	24–36	37	17	46	14	32
Present experiments	8.6 Millicurie-seconds	1.6×10^{12}	2–3½	66	39	59	3.3	56

It is also of interest to note that this study of sterility is in agreement with GEIGY'S results with ultra-violet light in confirming the work of embryologists on the origin of the reproductive cells. It is at present supposed, on the basis of somewhat indirect evidence, that these cells are set aside at between the eighth and twelfth cleavage, and that all subsequent reproductive cells are derived from these parent cells. This result is confirmed because permanent sterility is produced in the present experiments by damaging these parent cells, although all other cells from which germ cells might originate were protected.

COMPARISON BETWEEN ALPHA-RAYS AND X-RAYS IN PRODUCING LETHAL MUTATIONS

In this section a comparison is made between the alpha-ray dosages used in the present experiments and the X-ray dosages which have been employed by various observers for producing mutations with X-radiation. In the case of X-rays (and also gamma rays) it is at present well established that the dosage, or the amount of treatment to which the mutation effects are proportional, is determined by the total number of ions per cubic centimeter, which are produced by the X-rays in air at the position of the irradiated material. This quantity, which is equivalent to the rate of ionization multiplied by the time of treatment, is thus proportional to the total energy absorbed by the fly material during the treatment. Various

dosages have been used in the X-ray work. A representative treatment, which I adopt for the comparison, is that used by MULLER. It corresponds to 7×10^{12} ions per cubic centimeter.

For the alpha-ray treatment the average dose was about ten millicurie-seconds. It is now necessary to calculate the amount of ionization to which this corresponds. It is known that the number of alpha-particles emitted per second from one millicurie of polonium is 3.7×10^{7}. These are sent out in all directions. The number passing through a small area at the position of the egg, per unit area, is $\frac{3.7\times10^{7}}{4\pi(0.42)^{2}}$, or 1.7×10^{7}, since the eggs were rayed at a distance of 0.42 cm from the source. This figure is of course only approximate since the source extended over an appreciable area, but it is sufficient for the present purpose. Thus, about 1.7×10^{7} alpha-particles per second are passing through a cubic centimeter of air at the position of the eggs. Each alpha-particle at this point in its path is known to produce about 2.8×10^{4} ions per centimeter of path. Therefore, the number of ions per cubic centimeter per second is 1.7×10^{7} times 2.8×10^{4}, or 4.8×10^{11}. This figure is for one millicurie for one second. For one millicurie for ten seconds, or ten millicurie-seconds, the ionization is $10\times4.8\times10^{11}$, or 4.8×10^{12}. It is seen that the ionization for the alpha-ray treatment turns out to be of the same order of magnitude as the value 7.2×10^{12} of the ionization used by MULLER.

A parallel comparison may be made of the lethal mutation rate in the two types of experiments. MULLER reports finding about fifteen lethal mutations per one hundred treated germ cells. In the present experiments, using the results of the final experiments, I find seven lethals which were definitely caused by the treatment in 149 eggs. Since, however, each egg contains thirty cells on the average, the number of cells which were treated is 149×30, or 4470. Thus the number of mutations per treated cell is seven in 4470, or one in 640. The alpha-ray rate is seen to be much lower than that due to X-rays for comparable treatment. This is probably caused by the concentrated character of the alpha-ray ionization as compared with the more or less uniform distribution of the ionization due to X-rays. The effect of this concentration is to lessen the chance of producing the proper ionizing or chemical effects at the proper spot for producing a mutation, though the total ionization is the same.

SUMMARY

Seven "re-duplicated" lethals were obtained from 149 undechorionated eggs, treated at the polar-cap stage with alpha-radiation with a dose between one and six-tenths (1.6) and ten millicurie-seconds. The treatment increased the "natural" mutation rate 4,600 times during the time rayed.

From ninety-two dechorionated eggs, treated at the polar-cap stage with alpha-radiation with a dose between two and five millicurie-seconds, with the somatic cells adequately shielded, one "re-duplicated" lethal and one "re-duplicated" sex-linked visible wing mutation were obtained.

In thirty-six eggs treated at the polar-cap stage with a dose between 1.4 and 2.4 millicurie-seconds with alpha-radiation, without adequate shielding of the somatic cells, no "re-duplicated" lethals were produced.

Seven percent of the flies from the treated eggs, in the experiments on translocations, showed somatic variations.

No translocations were produced in eggs using a dose of five millicurie-seconds or less, but it is possible that they would be produced with better shielding and a bigger dose. However, no inversions were produced in any of the eggs of the experiments for the production of lethals. This was checked by the crossing over in the linkage counts for locating the lethals.

The percent of sterility produced by alpha-rays is higher than the amount produced by X-rays, and indications are that it would be considerably higher with an alpha-ray dose equal to the X-ray dose.

This study of sterility is in agreement with GEIGY'S results with ultra-violet light in confirming the work of embryologists on the origin of the reproductive cells. It is at the present supposed, on the basis of somewhat indirect evidence, that those cells are set aside between the eighth and twelfth cleavage, and that all subsequent reproductive cells are derived from these parent cells. This result is confirmed because permanent sterility is produced in the present experiments by damaging these parent cells, although all other cells from which germ cells might originate were protected.

The two cases of "re-duplicated" lethals, in which 100 percent of the offspring contained the lethal also confirm the study of the embryologist in this matter.

The alpha-ray mutation rate (one lethal per 640 germ cells) is much lower than that due to the X-rays (15 lethals per 100 germ cells) for comparable treatment. This is probably caused by the concentrated character of the alpha-ray ionization as compared with the more or less uniform ionization due to the X-rays. The effect of this concentration is to lessen the chance of producing the proper ionizing or chemical effect at the proper spot for producing a mutation, though the total ionization is the same.

An improvement was made in the technique of treating eggs at the polar-cap stage in that a sample of the eggs to be treated was examined under the microscope for the polar-cap stage just previous to the time of treatment.

LITERATURE CITED

ALTENBURG, E., 1928 The limit of radiation frequency effective in producing mutations. Am. Nat. **32**: 540–545.

1933 The production of mutations by ultra-violet light. Science **78**: 587.

CHILD, G. P. and HOWLAND, R. B., 1933 Observations on early developmental processes in the living egg of *Drosophila melanogaster*. Proc. Science **77**: 396.

GEIGY, RODOLPHE, 1931 Action de l'ultra-violet sur le pole germinal dans l'oeuf de *Drosophila melanogaster*. Université de Geneve Thése No. 895.

HANSON, F. B. and HEYS, F. M., 1928 The effects of radium in producing lethal mutations in *Drosophila melanogaster*. Science **68**: 115.

HANSON, F. B. and WINKLEMAN, E., 1929 Visible mutations following radium irradiation in *Drosophila melanogaster*. J. Hered. **20**: 277–286.

HEUTTNER, A. F. and RABINOWTIZ, M., 1933 Demonstration of the central body in the living cell. Science **78**: 367.

MOORE, W. G., 1932 The effects of X-rays on fertility in *Drosophila melanogaster* treated at different stages in development. Biol. Bull. **66**: 294–305.

MULLER, H. J., 1927a Artificial transmutations of the gene. Science **66**: 84–87.

1927b The measurement of gene mutation rate in Drosophila, its high variability and its dependence upon temperature. Genetics **13**: 2.0–335.

1928 The production of mutations by X-rays. Proc. Nat. Acad. Sci. Washington **14**: 714–726.

MULLER, H. J. and MOTT-SMITH, L. M., 1930 Evidence that natural radioactivity is inadequate to explain the frequency of natural mutations. Proc. Nat. Acad. Sci. Washington **16**: 277–285.

STADLER, L. J., 1928 Genetic effects of X-rays in maize. Proc. Nat. Acad. Sci. Washington **14**: 69–75.

HUMAN TWINNING IN THE UNITED STATES: RACIAL FREQUENCIES, SEX RATIOS, AND GEOGRAPHICAL VARIATIONS

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INTRODUCTION

In reviewing recently the publications on human multiple twinning, I was struck by the scantiness of quantitative data bearing on the subject. Most writers quote VEIT's statistics on twinning in Prussia, collected seventy-five years ago, or give approximate frequencies of twinning without any citation of sources or authorities. Fortunately, however, there has been accumulating since 1915 a significantly large and constantly increasing mass of data on the subject of multiple births in the United States. These figures are to be found in the yearly reports on birth statistics issued by the Census Bureau. I have attempted in this paper to compile and analyze the data so as to make them readily available for use. There are certain obvious conclusions that may be drawn from the figures, but for the most part I have not tried to dilate upon possible theoretical deductions. In addition to the data for the United States, I have included for comparison a few statistics from other countries; the sources for these are listed in the bibliography.

The first annual report on birth statistics in the United States was issued in 1915, but the first seven reports did not include single still-births, nor multiple still-births where none of the children were alive at birth. I have accordingly begun with the report for 1922, in which complete data are given by sex, color, and state of registration for all births and still-births, and for both single and multiple births. Figures from thirty states were available that year, and by 1930 (the date of the current report) forty-six states and the District of Columbia were reporting. Slightly over eighteen million births were reported in the nine years.

There is an unfortunate defect in the reports on the colored births. The single births are listed for white, negro, Indian, Chinese, and Japanese separately, but the multiple births since 1922 have not been classified in this way but have been recorded merely as white or "colored." There is not much question as to the bearing of these figures on twinning in the negro race, for 94 percent of the "colored" births are negro, and only 6 percent Indian or Asiatic. I have checked the 1923–1930 figures on "colored" births against: (1) the figures for twinning in the negro alone, as

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given in the 1915–1922 reports; and (2) the figures for "colored" twins in some of the southern states where the negro births form more than 99 percent of all "colored" births; and I feel no hesitation in saying that the figures for multiple births in the "colored" population may be taken to represent the negro race. On the other hand, it is impossible to draw any conclusions from these data as to the relative frequency of twinning in Amerinds or Asiatics.

THE FREQUENCY OF TWINNING IN AMERICAN WHITES

The statistics on multiple births in the white population in this country are given in table 1. It will be seen that the frequency of multiple births is about the same as was found by VEIT in Prussia (1/89). Twins, of course, are vastly more common than cases of triplet or quadruplet birth. We may point out from this table a popular fallacy that has gone unquestioned for years as to the proportional relations between the num-

TABLE 1

Mutiple births in the registration area of the United States between 1922 and 1930, in members of the white race. Includes both live- and still-births.

	ALL PREGNANCIES	ALL MULTIPLE BIRTHS	TWINS	TRIPLETS	QUADRUPLETS
Number	16,538,001	189,358	187,499	1,829	30
Percent of all pregnancies		01.145	01.133	00.011	00.00018
Fractional frequency		$\frac{1}{87.3}$	$\frac{1}{88.2}$	$\frac{1}{9042}$	$\frac{1}{551,266}$

bers of twins and of triplets. It has been said that if $\frac{1}{n}$ equals the frequency of twin births, then $\frac{1}{n^2}$ will be the frequency of triplet births, and $\frac{1}{n^3}$ that of quadruplets. From the above table, we see that $\frac{1}{n}$ is $\frac{1}{88.2}$. $\frac{1}{n^2}$ then, should be $\frac{1}{7779}$, and $\frac{1}{n^3}$ should be $\frac{1}{682807}$. Comparing these with the actual values in the table, it is seen that triplet births are 14 percent less frequent, and quadruplets 19 percent more common than the alleged formula would lead us to expect. We must conclude that the $\frac{1}{n}:\frac{1}{n^2}$ ratio between twin and triplet births is only an approximation, and not a very close one at that.

It must not be concluded that the frequency of twinning as found in this country will apply to all other nations. It has always been pointed out that there are variations in this respect from one country to another.

TABLE 2

Frequency of multiple births in American whites and in certain other nations.

NATION	NUMBER OF BIRTHS	MULTIPLE BIRTHS	FREQUENCY
U. S. White	16,538,001	189,358	$\frac{1}{87.3}$
Ontario	69,209	793	$\frac{1}{87.3}$
Australia	117,258	1,271	$\frac{1}{92.3}$
New Zealand	27,316	343	$\frac{1}{79.6}$
South Africa (Europeans)	46,423	545	$\frac{1}{85.2}$
Belgium	665,355	11,959	$\frac{1}{55.6}$
Uruguay	33,709	389	$\frac{1}{86.7}$
Norway	1,693,202	24,493	$\frac{1}{69.2}$
Sweden	Percent of multiple births, 1851–1920 varied between 01.425 and 01.499		$\frac{1}{68 \pm 2}$

A few illustrative figures are given in table 2. There is seen to be a high frequency of twinning in Scandinavia, a fact which has been noted many times previously, and an even higher percentage in Belgium. It is interesting to note that twinning seems to be of ordinary frequency in Uruguay, as some writers have claimed that multiple births are usually rare in Mediterranean racial stocks.

FREQUENCY OF TWINNING IN "COLORED" (=NEGRO) RACES

The data given in table 3 are the Census Bureau's figures for colored births; as was pointed out previously, these are composed of approximately

TABLE 3

Multiple births in the registration area of the United States between 1922 and 1930, in members of the "colored" races. Includes both live- and still-births.

	ALL PREGNANCIES	ALL MULTIPLE BIRTHS	TWINS	TRIPLETS	QUADRUPLETS
Number	1,829,077	26,336	25,977	352	7
Percent of all pregnancies		01.44	01.42	00.19	00.00038
Fractional frequency		$\frac{1}{69.5}$	$\frac{1}{70.4}$	$\frac{1}{5196}$	$\frac{1}{261,297}$

94 percent negro, 2 percent Indian, and 4 percent Asiatic births. Multiple births are seen to be 25 percent more frequent than in United States

whites, and the excess of triplet and quadruplet births alone is even greater. As in the white births, the alleged rule of $\frac{1}{n}:\frac{1}{n^2}$ is seen to give a false idea of the proportion of twin to triplet births.

As stated previously, I feel that the above results should be taken as applying to the negro, and not to the "colored" races in general. I have at hand two definite statements as to twinning in these groups. ABERLE found 4 sets of twins in 644 pregnancies among the Pueblo Indians, an incidence of $\frac{1}{161}$; and LAUFFER quotes a French physician, Dr. A.-T. MONDIÈRE, as having records of 153,174 births among the Annamese with only 15 multiple births. If multiple births are really so infrequent among Amerinds and Asiatics, then the values of table 3 are somewhat lower than they would be if the table included only negro births. In any case, the values in the table may be looked upon as representing a minimum, and twinning in the negro is definitely more common than in the white.

It has been objected that the figures for the negro may be inaccurate because of incomplete reports from the southern states. It is quite probable that the reports are not always complete, although I doubt that the southern states as a whole are any worse in this respect than other parts of the country. Even where the reports are incomplete, we might expect that they would be near enough to a random sample to yield valid percentages. However, in order to check this point, I examined the returns on colored births from several of the states separately. There seems to be some variation in the percentage of multiple births from one state to another, but this variability is not nearly as great as the similar fluctuations in the white births, nor does it seem to bear any relation to sectional lines, for in most of the northern states with a large negro population the percentage of multiple births is as high as it is in the south. It seems certain that the differences shown between whites and negroes in tables 1 and 3 represent real differences of a racial character.

SEX RATIOS IN MULTIPLE BIRTHS, AND THEIR SIGNIFICANCE

In table 4 I give the distribution of the sexes in all cases of multiple births and still-births reported since 1915. This table enables us to establish two points. The first of these concerns the sex ratios in multiple births as compared with that of births in general. Considering first the sets of twins, we find their sex ratio to be 103.5 for the whites, and 100.6 for the colored. For all births between 1922 and 1930 the ratios were: white, just under 106; colored, slightly over 103. There is thus a definite excess of females in twins as compared to the ratio in single births. In the triplet births, there is an absolute as well as a relative preponderance of females;

TABLE 4

Sex ratios in multiple births in the registration area of the United States, 1915–1930. For the years 1915–1921 the figures include only cases where at least one member was alive at birth.

	TWINS		
	♂♂	♂♀	♀♀
White	95,685	92,670	90,892
Colored	10,847	11,916	10,745

	TRIPLETS			
	♂♂♂	♂♂♀	♂♀♀	♀♀♀
White	711	649	697	726
Colored	103	100	103	155

	QUADRUPLETS				
	♂♂♂♂	♂♂♂♀	♂♂♀♀	♂♀♀♀	♀♀♀♀
White	13	6	8	6	7
Colored	0	0	4	1	3

the ratio falling to 97.7 for white and 79.4 for colored. The numbers of quadruplets are hardly large enough to be reliable. In them there is a decided preponderance of males among the whites and of females among the colored.

From the sex ratios and the distribution of the sexes in the twin pairs, we can now calculate the relative frequencies of fraternal and identical twinning. If the sex ratio in the white twins is 103.5, then the distribution of pairs of fraternal twins will be $(103.5)^2$♂♂, 2(103.5) (100)♂♀, and $(100)^2$♀♀. Since all the ♂♀ pairs are fraternal twins, the actual number (92,670) of these being known enables us to calculate the numbers of ♂♂ and ♀♀ pairs which are fraternals, and by subtraction to obtain the numbers of like sexed pairs which are identicals. Solving in this way, we find that out of a total of 279,247 sets of twins about 93,827 pairs, or slightly over 33 percent, must have been identical. Similar calculations indicate a little less than 29 percent of identicals in the colored twins.

	♂♂	♂♀	♀♀
twin pairs	95,685	92,670	90,892
fraternal pairs	48,000	92,670	44,750
identical pairs	47,685		46,142

The above figures differ from those of NICHOLS, who found only 26 percent of twin pairs to be identicals. NICHOLS also found a reduction in the

sex ratio in multiple births, but not to such a marked degree in the twin and triplet births as is shown in this paper. On the other hand, he found a sex ratio of only 54.8 in quadruplet births, while in the cases listed here there is actually a preponderance of males in white quadruplets. These differences are possibly due in large measure to the fact that NICHOLS' data were compiled from all the available records of various nations in both Europe and the Americas, and hence was of different racial composition, and of even less homogeneity, than the data presented here. As a possible bit of evidence that the tendency to the occurrence of multiple births differs in our series, I may point out that quadruplets occurred three times as often in my records as in NICHOLS'.

REGIONAL DIFFERENCES IN THE INCIDENCE OF TWINNING

The regional variations in the frequency of multiple births in the white race are shown in table 5 and figure 1. The negro has not been considered in this connection because of the impossibility of separating records of Indian and Asiatic births from those of the negro. In as far as they can

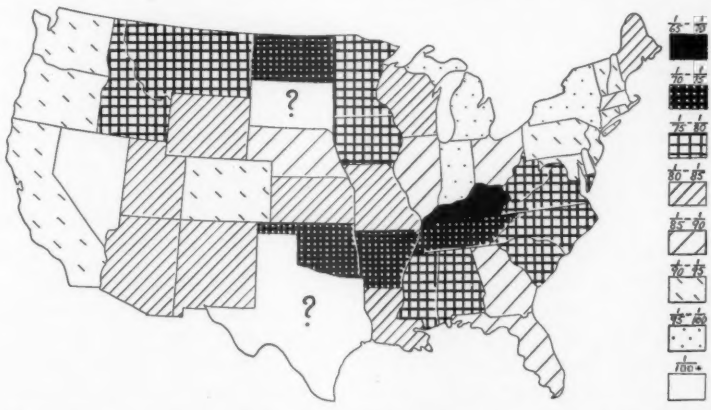

FIGURE 1.—Map to show the incidence of multiple births in whites by states, compiled fro the data given in table 5. The basis used is the fractional frequency, that is, one multipl birth in so many pregnancies.

be interpreted, however, the data indicate that the negro is less variable than the white in the frequency of twinning, although some states do differ in this respect.

It is evident from the map in figure 1 that there are two regions in which twinning is exceptionally frequent, one in the south and one in the northern plains region, but the reasons for these differences are obscure. I feel that these variations in the frequency of multiple births are probably due

TABLE 5

Frequency of multiple births in whites, by State of registration. Includes all data available from 1922 to 1930.

STATE	TOTAL BIRTHS	MULTIPLE BIRTHS	FREQUENCY (1 IN:)
Alabama	170,490	2,175	78.2
Arizona	38,553	462	83.4
Arkansas	124,187	1,769	70.3
California	701,498	7,449	94.4
Colorado	56,262	603	93.3
Connecticut	264,508	2,898	91.3
District of Columbia	50,007	524	95.4
Delaware	34,778	326	106.6
Florida	149,026	1,691	88.1
Georgia	113,003	1,324	85.4
Idaho	45,352	568	79.8
Illinois	1,166,547	13,040	89.4
Indiana	557,865	5,701	97.8
Iowa	319,024	4,019	79.4
Kansas	317,476	3,748	84.7
Kentucky	468,365	7,006	66.8
Louisiana	109,254	1,346	81.2
Maine	147,153	1,785	82.4
Maryland	246,488	2,634	93.5
Massachusetts	728,548	8,301	87.7
Michigan	872,533	9,003	96.9
Minnesota	473,639	6,184	76.5
Mississippi	208,012	2,654	78.3
Missouri	244,973	2,941	83.2
Montana	88,432	1,156	76.5
Nebraska	259,451	3,007	86.5
Nevada	2,474	20	123.7
New Hampshire	83,253	910	91.4
New Jersey	629,260	6,725	93.6
New Mexico	22,793	280	81.3
New York	2,019,614	20,820	97.0
North Carolina	518,646	6,730	77.0
North Dakota	102,577	1,382	74.2
Ohio	1,085,930	12,644	85.9
Oklahoma	119,050	1,610	74.0
Oregon	130,568	1,430	91.3
Pennsylvania	1,830,665	19,870	92.2
Rhode Island	137,867	1,212	113.8
South Carolina	134,353	1,715	78.4
Tennessee	179,208	2,430	73.7
Utah	118,974	1,459	81.6
Vermont	65,817	720	91.4
Virginia	381,930	4,828	79.1
Washington	210,175	2,331	90.2
West Virginia	250,327	3,180	78.7
Wisconsin	521,371	6,226	83.8
Wyoming	41,625	515	80.8

in large part to racial differences; and such differences are known to exist among European populations; but our knowledge of the racial stocks involved in the present case is too indefinite to allow us to do more than guess at the possibilities. The high percentage of twinning in Minnesota and North Dakota might be due to a large Scandinavian element in these states (see Norway and Sweden, in table 2), but I doubt that this explanation could be extended to Montana and Idaho. Similarly, if we try to explain the frequent multiple births in the southern states as being a racial character of the predominant "old American" stock, why then does not Georgia, with a similar population, agree with them in this respect? It is known that the frequency of twinning increases with the size of the family and the age of the parents (this is pointed out in the Census reports), and it is possible that these factors may be concerned in some way. I feel, however, that our present knowledge is too inadequate to allow us to draw any valid conclusions as to the causes of this remarkable regional fluctuation in twin production; and I prefer to avoid adding any more unverifiable speculations to the vast store with which the whole subject of human twinning is already encumbered.

APPENDIX

An important paper dealing with this same subject came to my attention after this manuscript was already in the editor's hands. This reference is: Sir K. DAS, 1934, Twin pregnancy (a demographic and ethnic study), J. Obst. Gyn. Brit. Emp. **41:** 227–255. DAS'S statistics are from varied sources and are of uneven value. He includes the figures for the United States from 1915 to 1929, giving frequencies of 1/88 for whites and 1/67 for colored. His figures for Germany, Italy, Scotland and Japan are significantly large, the frequency of twinning being between 1/82 and 1/85 for the first three and 1/301 for Japan. The Japanese rate, based on 17 million births, is important as establishing the low twinning rate in Asiatics. Another interesting group of DAS'S figures deals with a series of 48 thousand births in Calcutta hospitals. Europeans and Eurasians show a twinning frequency of 1/95; full blooded Indians of 1/59. DAS naïvely brackets these figures for the Indians with those for United States negroes to prove a higher twinning for colored races against whites, a procedure which implies a total misconception of the idea of racial differences.

LITERATURE CITED

ABERLE, S. B. D., 1932 Child mortality among Pueblo Indians. Amer. J. Phys. Anthr. **16:** 339–349.

AUSTRALIA, Official year-book for 1932.

BELGIUM, Annuaire statistique de la Belgique, 1929–1930.

Mondière, A.-T., 1875 Monographie de la femme Annamite. Mem. de la Soc. d'anthrop., t. 2, pp. 474– (cited in "Multiple births among the Chinese," B. Laufer, Amer. J. Phys Anthr. 3: 83–96).

New Zealand, Official year-book for 1932.

Nichols, J. B., 1907 The numerical proportions of the sexes at birth. Mem. Am. Anthrop. Ass 1: 249–300.

Norway, Statistisk Ärbok for Kongeriket Norge, 1929.

Ontario, Report relating to the registration of births, marriages, and deaths in the Province of, 1931.

South Africa, Official year-book, for 1931–1932.

Sweden, Statistist Ärsbok for Sverige. 1929.

United States, Birth, still-birth, and infant mortality statistics. Annual reports, 1915–1930. U. S. Dept. Commerce, Bureau of the Census.

Uruguay, Anuario Estadistico de la Rep. Orient. del, 1904–1906.

Veit, G., 1855 Beiträge zur geburtshülflichen Statistik. Monats. f. Geb. u. Frauenk., 6: 126–132.

THE GENETICS OF X-HYPERPLOID FEMALES*

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This is the first of a series of papers in which we propose to discuss the question of genic balance in its relation to the X chromosome in *Drosophila melanogaster*. For several years we have been interested in studying the genetic effects of pieces of the X chromosome produced by X-radiation. This method of experimentation gives opportunity to investigate the genetic effects on the zygote of adding or subtracting pieces of various lengths and of known constitution from different regions of the chromosome. The present paper deals with the effects of extra pieces on the phenotypic expression, viability, and fertility in X-hyperploid females.

The stocks upon which this study is mainly based were obtained in an experiment dealing with the production of translocations (PATTERSON, STONE, BEDICHEK, SUCHE 1934). From the large number of translocations secured, we have selected several cases best suited to our purpose, in which a piece of the X chromosome is attached to the IV chromosome, or to the II chromosome, or to both. These stocks, together with three others that were available in the laboratory, form a series in which the X chromosome had been broken at rather regularly spaced intervals along its length. The reason for selecting chiefly X-IV translocations is because most translocations are mutuals, and the small piece of the IV chromosome attached to the X causes less disturbance in the X-hyperploids than the usually longer piece from one of the larger autosomes.

DESCRIPTION OF STOCKS

Seven of the nine cases selected from our translocation stocks represent simple breaks in the X chromosome, with the left-hand piece attached to the IVth. All nine of these cases are listed in table 1, where the terms by which they are designated in the text are given in the first column. The second column indicates the point, or points, of breakage in the X; the third, the point of breakage in the IV, where this is known; the fourth, the designations of these stocks in the literature; and the last column, under remarks, gives further facts of interest.

Stock M5 is a white mottled stock, found by MULLER several years ago. Stock w13, also found by MULLER, is interpreted by him as an insertion of the vermilion-carnation-bobbed section of the X chromosome into the IV chromosome, in the inverted order, so that vermilion is now near the

* Part of the cost of the accompanying illustrations is paid by the Galton and Mendel Memorial Fund.

spindle fiber of the IV. As this section includes all the known genes in the right-hand and of the X, the fact that it is an insertion is irrelevant for our purpose. Stock 1 was found by STONE and has been used in several different investigations. All of the others were obtained by the authors.

TABLE 1

STOCK	BREAK IN X	BREAK IN IV	NOMENCLATURE IN THE LITERATURE	REMARKS
M5	*w-fa*	*ey-bt*	X-IV, 2, MULLER and PAINTER 1932	Single break in X
9	*rb-cv*	*eybt*-sp.f	TA1-4, 9, PATTERSON et al 1934	Single break in X
w13	*lz-v*	*eybt*-sp.f	X-IV, 1, MULLER and STONE 1930	Single break ?
8	*m-g*	*eybt*-sp.f.	TA1-4, 8, PATTERSON et al 1934	Single break
4	*sd-f*	left of *ey*	TA1-4, 4, PATTERSON et al 1934	Single break
1	*f-B*	left of *ey*	TA1-4, 1, PATTERSON et al 1934 X-IV, 4, MULLER and PAINTER 1932	Single break
13	*fu-car*	left of *ey*	TA1-4, 13, PATTERSON et al 1934	Single break
124	*lz-v, g-pl*	..	TA1-2, 124, PATTERSON et al 1934	Deletion, *v-g* to II
12	*sc-br, g-sd*	..	TA1-4, 12, PATTERSON et al 1934	Double deletion *ct* locus to II

Stock 124 has the *v-g* section attached to the II chromosome, while the *y-ct* and *pl-bb* sections are reunited as an independent element. Stock 12, as is indicated in the table, is more complicated. The *br-g* section is attached to the IVth, with the cut locus removed by a second deletion and attached to the II chromosome.

The X-hyperploid females from the simple breaks will be referred to as L and R, depending upon whether they contain the left or right-hand piece in duplication. The 124 X-hyperploid female with the *v-g* duplication is designated as 124M, while the one having the reunited ends in duplication is called 124 L+R.

With reference to the complicated case 12, it may be stated that five different kinds of X-hyperploid females are produced, all of which can be detected in the F_1 generation of the cross between the males with the translocation and females with attached X's and homozygous for yellow echinus cut vermilion garnet forked. We are especially interested in the *ec ct v g* and *y f* females, for these represent the equivalent of L+R and M types, respectively, and will be so referred to. Since the cut locus is attached to the II chromosome, and therefore undergoes independent segregation, three other classes of X-hyperploids are formed, namely, *y ec v g f*, *ec v g*, and *y ct f*.

All except stock 13 were carried by mating the males with the translocation to the *y-x*-ple females (*y ec ct v g f*). In order to be able to detect the X-hyperploid females with the *car-bb* duplication, it was necessary to use females in which the attached-X chromosomes were homozygous for carnation, and scute vermilion forked carnation females were therefore employed.

EXPERIMENTAL RESULTS

The results obtained in the tests for viability and fertility of the nine translocation stocks are presented in table 2. The third column of this table gives the first 1000 females counted for each stock, and shows in each instance the number of non-hyperploid females (*y-x*-ple in all except 13, which is *sc v f car*) followed by the X-hyperploids with the left- and right-hand duplications, respectively. The designation of the classes in Stocks 124 and 12 has been indicated above.

In making the crosses for the viability tests, care was taken not to overpopulate the culture bottles, which contained food rich in yeast. Furthermore, the counts were made each day from the time the flies began emerging until just before the F_2 generation was due to appear. This was done through a series of bottles until the desired count of 1000 females was reached. This method of procedure gives the hyperploids a chance to appear in case they are viable, for, like triple-X females, they usually emerge rather late in the culture.

The fourth column of the table gives the percentage of viability for the different classes. These percentages were determined by placing the non-hyperploids at 100 percent, and then from this calculating those for the X-hyperploids.

Columns five to eleven deal with the data on fertility. In the test for fertility, all classes of females were mated to normal males, one female and three males being placed in each culture vial. The character and quantity of food and temperature were kept as constant as possible. The experiment was conducted in an air-cooled room in which the temperature varied not over three degrees (74–77). For the first three days after making the matings, the vials were placed on their sides, in order to prevent as much as possible the loss of flies from sticking to the food and drowning. At the end of this period the vials were placed upright, and those in which the female had died were set aside to see if any proved to be fertile cultures. The few that were found to be fertile are included in the number that are listed as "tested" (column 7). The percent sterile (column 8) was then easily determined. The flies were etherized and counted consistently at the end of the thirteenth day after making the matings. Attempts were made to obtain tests on 100 females for each class, but this was found to be impracticable for certain of the inviable classes.

TABLE 2

STOCK	CLASS	RATIO	PERCENT OF VIABILITY	MATED	PERCENT SURVIVED	TESTED	PERCENT STERILE	♀♀	AV. ♀♀ PER VIAL	INDEX OF FERTILITY
	y-x-ple	676	100.0	110	90.9	100	8.0	1165	12.7	100.0
M5	L	323	47.8	112	89.4	100	22.0	413	5.3	41.7
	R	1	0.1	1	0.0	0	..	..	..	..
	y-x-ple	695	100.0	128	78.1	100	7.0	1162	12.5	100.0
9	L	304	43.7	152	65.7	100	38.0	387	6.2	49.6
	R	1	0.1	0	..	..	..	..	..	..
	y-x-ple	695	100.0	112	89.3	100	6.0	1225	13.0	100.0
w13	L	297	42.3	166	60.2	100	79.0	106	5.0	38.5
	R	11	1.6	22	45.5	10	90.0	2	2.0	15.4
	y-x-ple	768	100.0	121	82.6	100	10.0	1171	11.9	100.0
8	L	202	26.3	178	56.2	100	93.0	7	1.0	8.4
	R	31	4.1	36	58.3	21	76.2	3	0.6	5.0
	y-x-ple	606	100.0	118	84.7	100	12.0	1276	14.5	100.0
4	L	51	8.2	34	79.4	27	100.0	..	..	..
	R	343	56.6	128	78.1	100	39.0	577	9.5	65.5
	y-x-ple	430	100.0	119	84.0	100	12.0	1257	14.3	100.0
1	L	14	3.3	15	73.3	11	100.0	..	..	..
	R	556	129.3	141	70.9	100	30.0	490	7.0	40.6
	sc v f cr	344	100.0	120	83.3	100	10.0	1629	18.1	100.0
13	L	3	0.9	4	0.0	..	..	..	..	..
	R	635	192.7	115	87.0	100	8.0	2522	27.4	151.4
	y-x-ple	545	100.0	102	98.0	100	8.0	1619	17.6	100.0
124	L+R	33	6.1	73	49.3	36	100.0	..	..	..
	M	422	77.6	109	91.7	100	29.0	568	8.0	45.5
	y-x-ple	313	100.0	107	93.5	100	8.0	1073	11.7	100.0
	L+R	83	26.5	74	77.0	57	42.1	152	4.6	39.3
12	M	19	6.1	15	33.3	5	100.0	..	..	..
	y ec v g f	393	125.6	103	97.0	100	12.0	1541	16.4	140.2
	ec v g	178	56.9	104	96.1	100	22.0	319	4.1	35.0
	y ct f	14	4.5	17	47.1	8	87.5	one♂	..	..

The fifth column shows the number of females it was necessary to mate in order to obtain the 100 for the fertility tests where this was practicable. The sixth column gives the percent that survived the three day period. The comparison of these survival percents of the non-hyperploid and hyperploid classes gives the relative vigor of females that succeeded in emerging.

The ninth column gives the total number of females for each class. The number of males, although determined, is not included, because, ex-

cept for non-disjunction, the male offspring would be hyperploid, and these in most cases do not survive. The females therefore give a better basis for comparison. The average number of females per culture is shown in column 10. Finally, in the last column is given in percents what we may call the index of fertility. This was calculated by placing the average number of non-hyperploid females at 100 percent. The index of fertility is a measure of the reproductive capacity of the females. There is no positive correlation between the index of fertility and an index of vigor which is the ratio of hyperploid survival to the non-hyperploid survival at the end of the three day period.

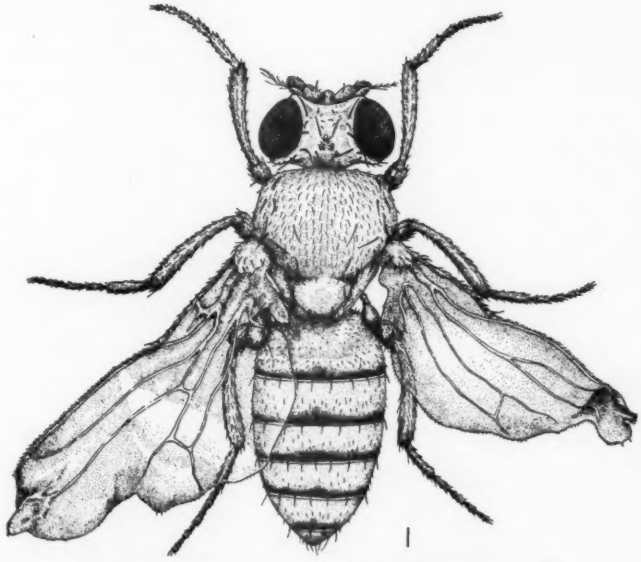


FIGURE 1.—A hyperdiploid female with the duplication M5R.

The following descriptions of the phenotypes of the X-hyperploid females are based on specimens obtained by crossing the translocation males to yellow vermilion forked females with attached-X's. From this the reader can tell which mutant characters are visible in any given hyperploid. Dissections were made of all the different kinds of hyperploids, and it was found that, with the exception of the ovaries, the external and internal reproductive organs were normal. When the percentage of fertility is referred to it means the corrected percent. The number of hyperploids examined in preparing the descriptions is given in parenthesis at the end of each paragraph.

Stock M5 (*w-fa* break). The M5L females are in general normal in appearance. They differ from their non-hyperploid sisters in having slightly narrower wings, bristles on the wing-veins, occipital bristles, and a few extra bristles on the head. The gonads are normal. These females are fairly viable (47.8 percent), are fertile to the extent of 84.8 percent, and have a fertility index of 41.7 (17).

The M5R females are very inviable. Only three specimens have been found among several thousand flies. Two of these were mated for the fertility test, but one died within the first day and the other proved to be sterile. The third specimen was used for the dissection. These hyperploids closely resemble superfemales (figure 1). The eyes are slightly rough, and the wings are abnormal and frayed at the margins. A few small bristles were found on the wing-veins of one of the females. The ovaries are small and undeveloped. It is probable that these females are entirely sterile (3).

Stock 9 (*rb-cv* break). The head of 9L females is normal in size and shape, but the eyes are usually reduced in size and are slightly rough (figure 2). In most females the thorax and abdomen are normal. The wings are narrower and longer than normal, with the posterior crossvein showing a small posteriorly directed branch, and the longitudinal veins terminating in deltas at the margin. The hind legs are often deformed. The bristles are thick with a few extra ones on the head or thorax. Occipital bristles are present and bristles on the wing-veins are common. The gonads are well-developed. These females are 66.6 percent fertile, but their fertility index of 49.6 shows that they are only about one-half as productive as their non-hyperploid sisters (25).

The 9R females are very inviable and are distinctly smaller than the normals. The head is broad and the eyes are narrow and rough. The thorax is narrow and the abdomen slender, but not pointed (figure 3). The wings are abnormal and are usually scalloped. The bristles are thin and extra ones are sometimes present. Bristles on the wing-veins and occipital region are absent. The gonads are rudimentary and the eggs do not develop. These females are sterile as shown by recent tests (11).

Stock w13 (*lz-v* break). The head of *w*13L females is slightly narrower than normal, and the eyes are small, narrow, and rough (figure 4). The wings are long and narrow. Occasionally double bristles are present on head and thorax. A few occipital and wing-vein bristles are present, but they are not as numerous as in 9L females. The gonads vary considerably in size in different females, ranging from normal to not over half size. These females are not especially fertile (22.3 percent) and have a fertility index of 38.5 (7).

The w13R females are more nearly like normals, but the head and thorax are larger and broader, and the abdomen is sometimes pointed. The eyes

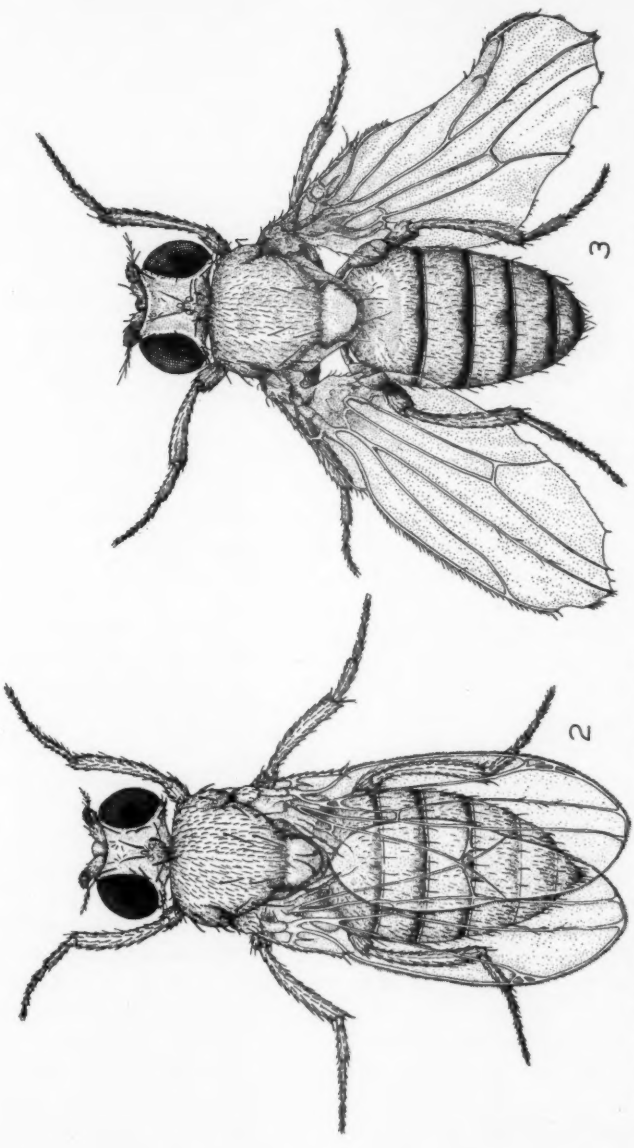


FIGURE 2.—A hyperdiploid female with the duplication 9L.

FIGURE 3.—A hyperdiploid female with the duplication 9R.

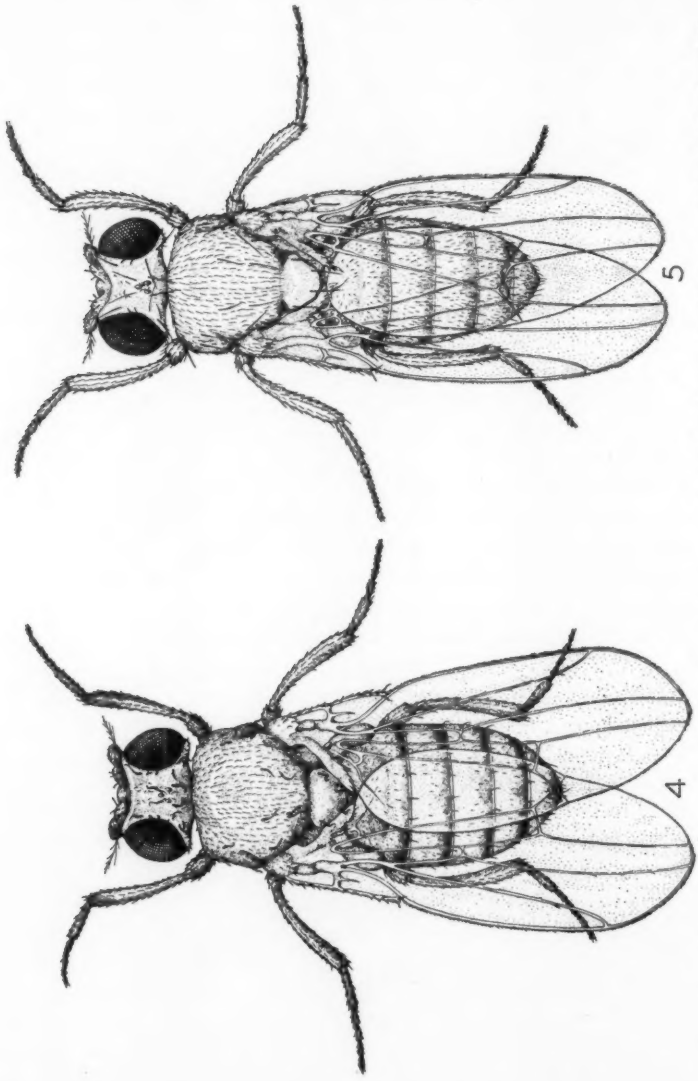


FIGURE 4.—A hyperdiploid female with the duplication w13L.

FIGURE 5.—A hyperdiploid female with the duplication w13R.

are narrower than normal and are slightly rough. The inner margins of the wings are thin and frequently scalloped (figure 5). The gonads are very poorly developed. These females are not very viable, but are sometimes fertile. Of the ten that were tested, only one proved to be fertile, and she gave but two female offspring (8).

Stock 8 (*m-g* break). The 8L females vary considerably in appearance, but in most cases resemble superfemales. The head is broad and the small eyes are extremely rough (figure 6). Both the thorax and abdomen are fairly normal in size and shape. The wings are nearly always misshapened, with veins sometimes thickened or otherwise abnormal. The third pair of legs may be distorted. Extra bristles are present on the thorax. A few occipital bristles are found, and still fewer wing-vein bristles. The latter when present are short and rudimentary. The gonad may be normal, but it is sometimes not over one-fourth as large as a normal ovary. These females are rarely fertile (7.8 percent) and have an extremely low fertility index of only 8.4 (16).

The 8R females (figure 7) are more normal in appearance than the 8L specimens and are also more fertile (26.7 percent). The wings are thin, especially at the inner margins, causing this margin to wrinkle. The wing-veins are sometimes thickened and frequently fail to reach the margin. The bristles are thin, but none is missing. The ovaries are poorly developed. Occipital and wing-vein bristles are absent. These females while more fertile than the 8L's, have a still lower fertility index, 5.0 (26).

Stock 4 (*g-sd* break). The 4L females (figure 8) resemble the superfemales and like them too are completely sterile. The eyes are small and rough, but not as rough as those of 8L. The abdomen is small, narrow, and usually pointed. The wings are often misshapened, and with thickened veins. A few extra bristles are found on the head and thorax, but occipital and wing-vein bristles are absent. The ovaries are rudimentary (4).

The 4R females are almost normal in appearance (figure 9). The bristles are rather thin with a postvertical sometimes missing. The gonads are underdeveloped. These females are 56.6 percent viable, 69.3 percent fertile, and have a fertility index of 65.5 (10).

Stock 1 (*f-B* break). The 1L females look like typical superfemales and are sterile. The head is broad with small rough eyes, and the thorax is foreshortened. The wings are misshapened with abnormal venation (figure 10). The legs are usually twisted and knotted. Extra bristles are present on head and thorax. The gonads are small with no mature eggs developed (3).

The 1R females are apparently entirely normal (Bar-eyed) but the bristles are slightly thinner than normal. These flies are more viable than normal (129.3 percent) but are only 79.5 percent fertile, with a fertility index of 40.6. The ovaries in most of the flies are fully developed (25).

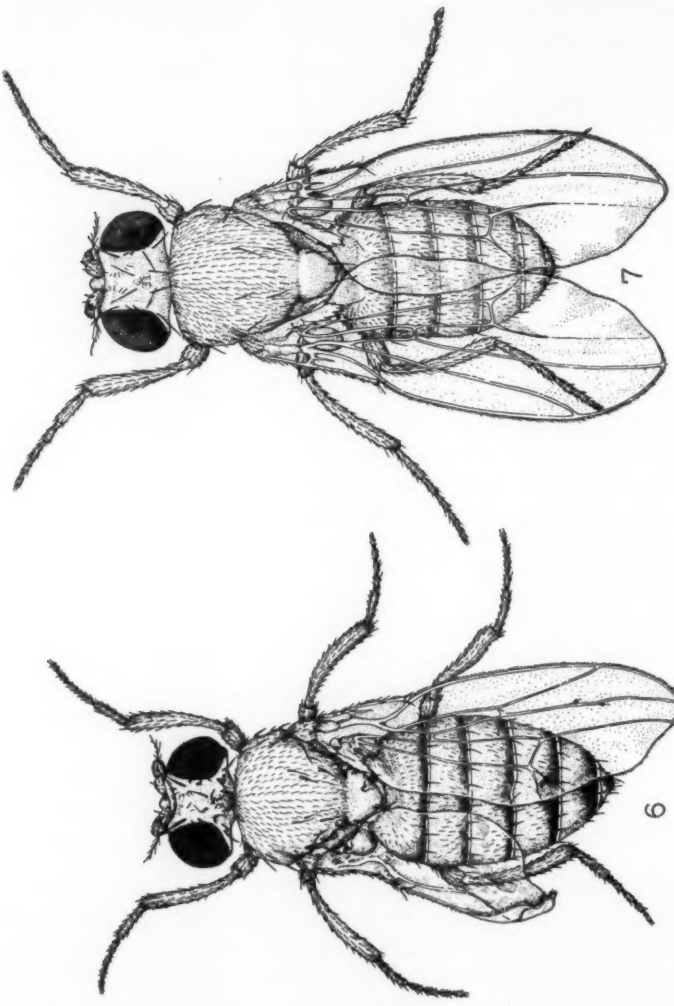


FIGURE 6.—A hyperdiploid female with the duplication 8L.

FIGURE 7.—A hyperdiploid female with the duplication 8R.

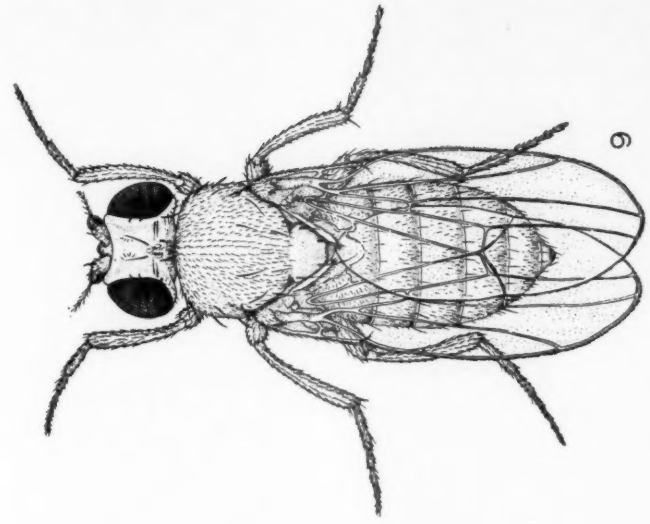


FIGURE 9.—A hyperdiploid female with the duplication 4R.

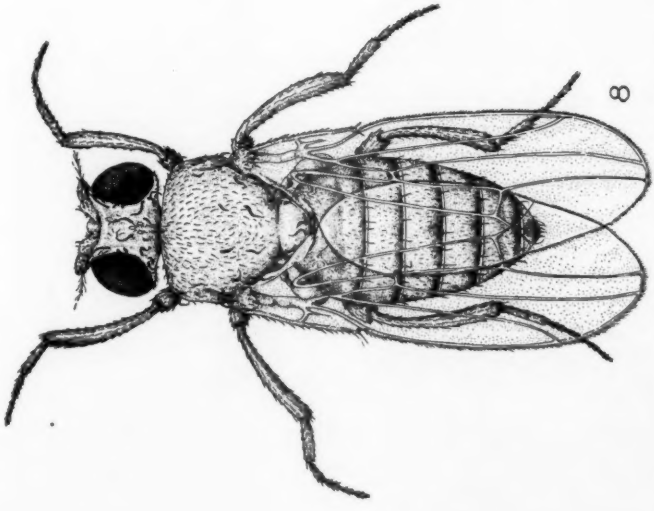
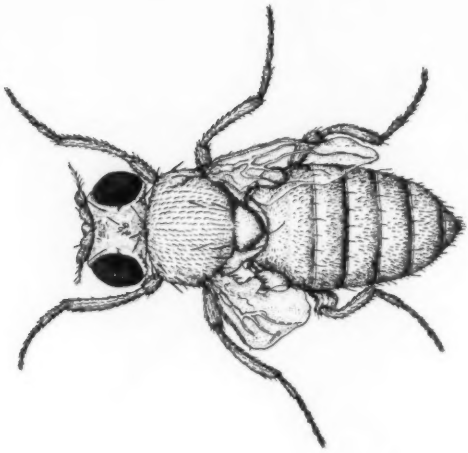


FIGURE 8.—A hyperdiploid female with the duplication 4L.



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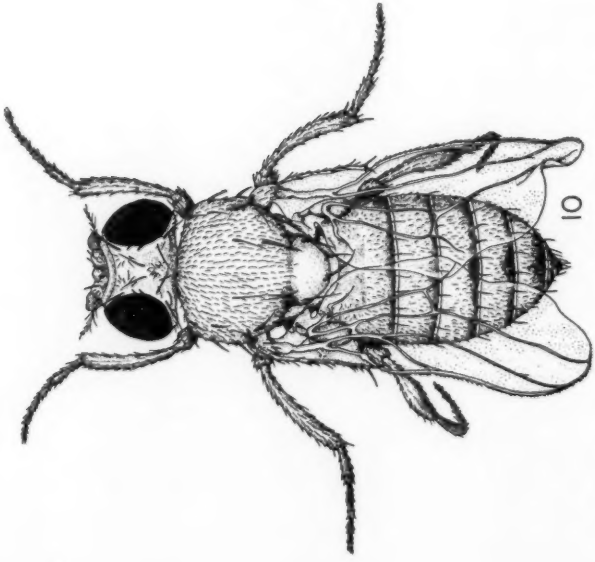

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FIGURE 10.—A hyperdiploid female with the duplication 1L.

FIGURE 11.—A hyperdiploid female with the duplication 13L.

Stock 13 (*fu-car* break). The 13L females are highly inviable and show the extreme superfemale type (figure 11). They are small, with rough eyes, abnormal wings, and twisted hind legs. Some of the bristles are missing. These flies were derived from the cross of translocation males to scute vermilion forked carnation females with attached X's. Their undeveloped gonads indicate that they are completely sterile (4).

The 13R females are entirely normal and have the very high viability percentage of 192.7. They are also slightly, though not significantly, more

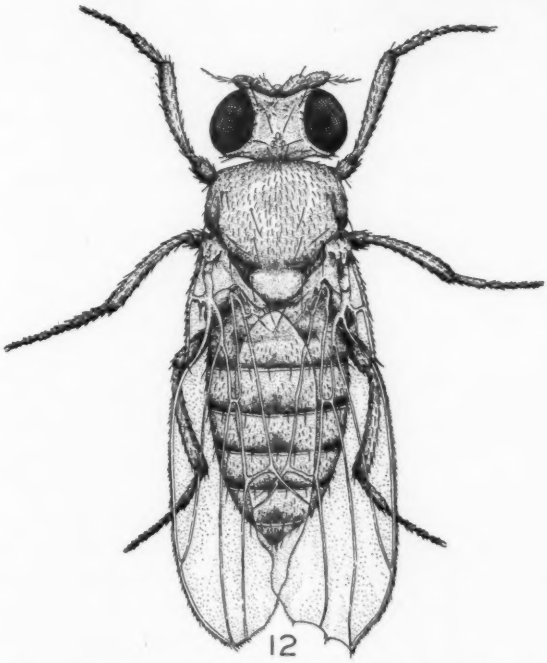

FIGURE 12.—A hyperdiploid female with the duplication 124L+R.

fertile than their non-hyperploid sisters. Their index of fertility is 151.4 (10).

Stock 124 (*v-g* deletion). The 124L+R females have small narrow rough eyes, and the thorax and abdomen are narrower than normal (figure 12). The wings are small and narrow and show a tendency to cup. The wing-veins are thickened and the margins are sometimes scalloped. Extra bristles are found on the head and thorax. Occipital bristles are absent, but a few wing-vein bristles are present. The ovaries are very rudimentary, and the flies are sterile (38).

The 124M females have the appearance of normal flies. The eyes are slightly more rounded than normal and the abdomen is not as broad. The gonads are usually well developed and the flies are fairly fertile (77.6 percent). They have a viability percentage of 76.7 and the index of fertility is 45.5 (12).

Stock 12 (double deletion). We shall describe but two of the five types of hyperploids found in this stock. The 12L+R females are nearly normal in appearance (figure 13). Extra bristles are present. These females are not especially viable (26.5 percent). The gonads are reduced in size, but they are 62.9 percent fertile and have a fertility index of 39.3 (5).

The 12M females are yellow forked and resemble superfemales (figure 14). The eyes are small and very rough. The abdomen is small, narrow and pointed. The legs are often twisted. The wings are narrow and usually abnormal. The veins are often thickened and show deltas at the margin. The postvertical and scutellar bristles are frequently missing. A very few occipital bristles are present. The ovaries are undeveloped but occasionally contain a few mature eggs. The few flies tested were sterile. However, a female of this type, but with the cut locus deficient (see last line of table 2) was found to be barely fertile, giving a single male offspring (19).

ANALYSIS OF THE DATA

In attempting to analyze the experimental data concerning the phenotypes of the different X-hyperploid females, one meets the difficulty of a lack of standards by which to measure quantitatively the various characters revealed. Obviously, the most logical method is to compare these flies with superfemales, which carry an extra X chromosome, but superfemales, although constituting a rather distinct somatic type, nevertheless show considerable variation. Their most outstanding characteristics are their inviability, poorly developed gonads, and consequent sterility.

Since the X-hyperploids from the different stocks carry X chromosome fragments of different lengths, it is possible to compare these females to superfemales as to the effect such fragments may have upon these characteristics. In general, one might expect to find that the longer the duplicating fragment the more nearly would the characters of the hyperploid approach those found in the superfemale. However, this would be true only on the assumption that each segment of the chromosome is like every other segment with respect to the genes influencing these characters. While our data show that this condition is approached in respect to the genes influencing fertility, yet they also demonstrate that certain phenotypical expressions are dependent upon limited regions of the chromosome.

The nature of the developmental effects of the different duplications has been brought out in the descriptions of the hyperploids given above. From

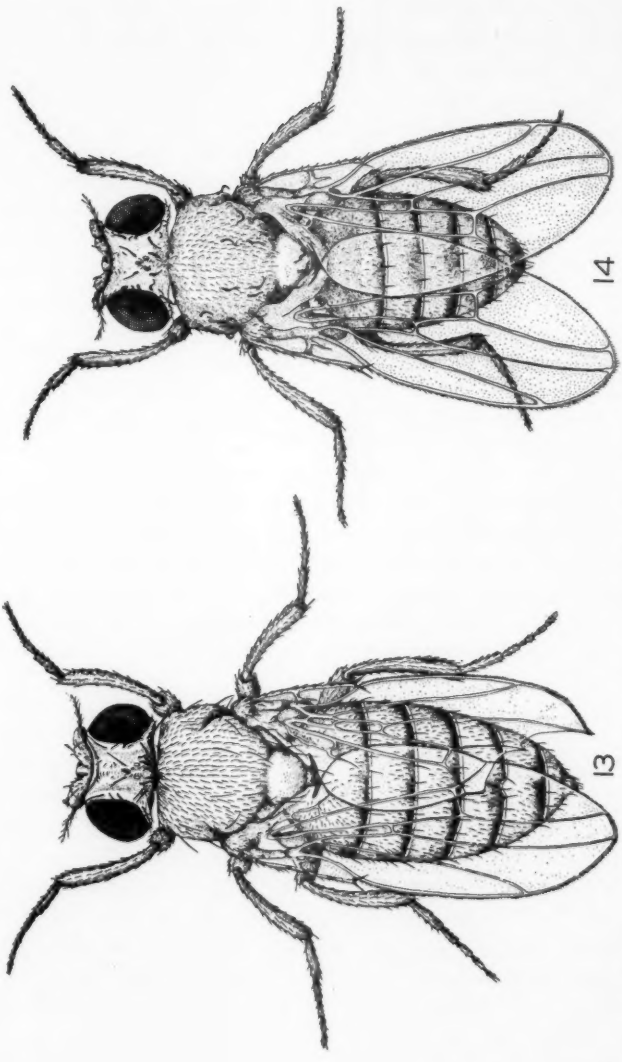

FIGURE 13.—A hyperdiploid female with the duplication 12L+R. FIGURE 14.—A hyperdiploid female with the duplication 12M.

these descriptions it is clear that while some characters are the result of summation effects, others evidently are due to regional difference in the chromosome. Such characters as rough eyes, occipital and wing-vein bristles, and narrow pointed wings are caused by duplications of the left-half of the chromosome and, in some instances, are limited to restricted regions of this end. Duplications of the right-end cause thin bristles and increase viability but do not bring about the appearance of rough eyes, narrow wings, or occipital and wing-vein bristles.

The outstanding visible effect of a summation character caused by duplications is the condition of the gonads. As longer and longer duplications from either end of the chromosome are introduced into the zygote, the ovaries become gradually reduced in size, until ultimately a point is reached at which they fail to function as gamete-producing organs. The physiological expression of this is the gradual reduction in fertility, leading up to complete sterility, which is the most distinctive characteristic of the superfemale.

The effect of adding pieces of the X chromosome in duplication on the fertility and viability of the resulting X-hyperploid females is very obvious from the data displayed in table 2. First, in the matter of fertility, if pieces of increasing length from the left-hand end be added, the percentage of fertility decreases. The same is true for similar pieces added from the right-hand end. This can be more clearly seen if the corrected percentages are used (obtained by dividing the percentage of fertility for the X-hyperploid class by that of its non-hyperploid class). For the left-hand pieces of the stocks containing simple breaks, the corrected percentages are as follows: M5, 84.8; 9, 66.6; w13, 22.3; 8, 7.8; and for stocks 4, 1, 13, 0.0. These percentages for the right-hand pieces, given in reverse order, are: 13, 102.2; 1, 79.5; 4, 69.3; 8, 26.7; w13, 10.6; and for stocks 9 and M5, 0.0.

These results are shown in graphic form in figure 15. The percentages represent the ordinates, while the exact points of breakage on the cytological map of the salivary-gland chromosome (after PAINTER) represent the abscissae, from which the curves were plotted.

The left and right fertility curves are not quite straight lines. If the left fertility curve were continued, it would meet the base line at a point lying to left of the abscissa for the break in stock 4; and this is correct, because 4L females are sterile. The same would be true for the right fertility curve, for it would meet the base line at some point lying to the right of the abscissa for break 9, and 9R females are sterile.

If these two curves can be interpreted as representing straight lines, it means that the rate of change in fertility is constant. In X-hyperploid females then, the percentage of fertility is inversely proportional to the length of the duplicating piece.

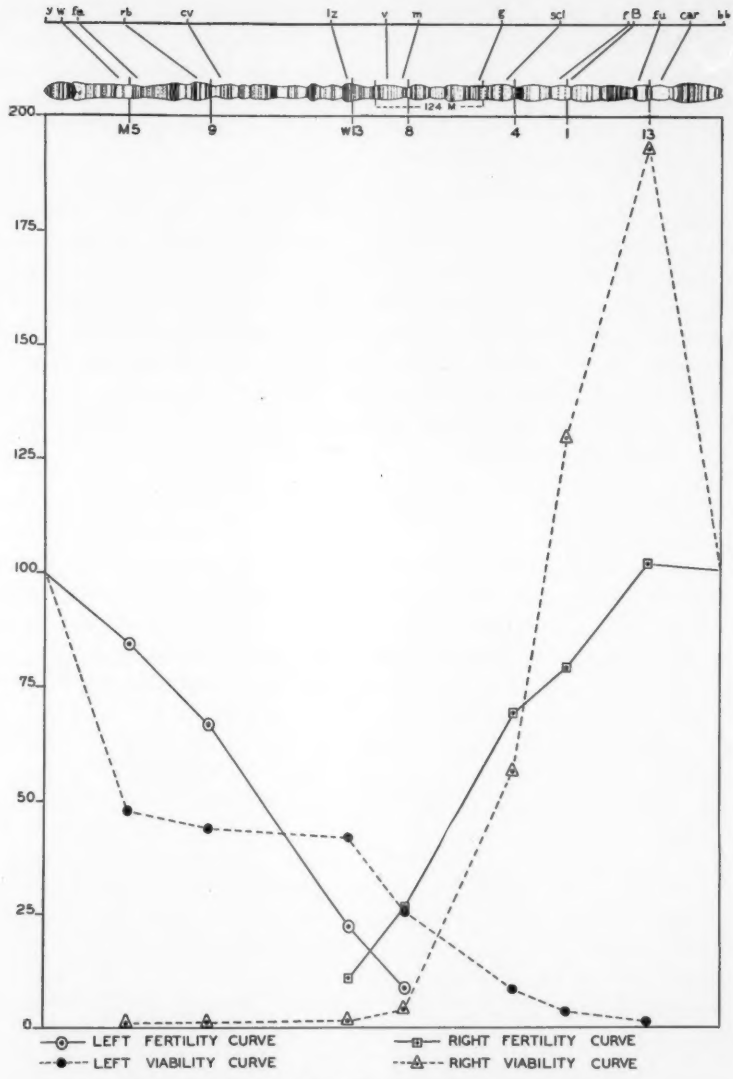

FIGURE 15.—A graph showing curves of fertility and viability.

Mr. OTTO MACKENSEN has kindly determined the points of breakage in stock 124, and these are indicated on the diagram.

The nature of the viability curves requires a different interpretation. The left curve starts at point M5, where it shows a decrease of 52.2 percent in the viability of the X-hyperploid females as compared to their non-hyperploid sisters. This indicates that the interval from yellow to the M5 break must contain genes which in duplication lower the viability more than fifty percent. Duplications extending beyond point M5 to as far as point w13 do not significantly lower the viability of the X-hyperploids, so that this interval must contain genes which but little affect viability. From point w13 to point 8 there is a sudden drop in the curve, amounting to a decrease of sixteen percent in viability. The genes in duplication lying between these two points very decidedly affect the viability of the X-hyperploids. From point 8 to point 4 the curve continues to drop sharply and then flattens out as it approaches the base line. It will of course not quite meet this line, because X-hyperploid females carrying an entire extra X (triple-X females) are occasionally viable, and it is to be expected that females with duplications that are somewhat less than the whole X chromosome would sometimes be viable.

The right viability curve stands out in sharp contrast to the left curve. The interval from point 13 to bobbed, when added in duplication, increases the viability of the X-hyperploid to almost double that of their non-hyperploid sisters (192.7 percent). This section of the chromosome includes the so-called inert region and either this is responsible for the enormous increase in vigor, or else certain genes lying near to the locus of carnation represent the active agency. This influence is also reflected in duplications that are longer than 13R, for X-hyperploid females with the interval from point 1 to bobbed also show a significant increase in viability of 29.3 percent above that for their non-hyperploid sisters.

If the right-hand piece is increased from point 1 to point 4, the viability is lowered to only 56.6 percent, representing a drop of 72.7 percent. The relatively short section lying between these two points must therefore greatly affect the viability of the hyperploids in a negative direction, counteracting the positive influence of sections lying nearer the right end. Further additions of longer right-hand duplications from the active genic region show this same counteracting influence. Thus, the viability of the 8R hyperploids drops to 4.1 percent. The addition of the interval from points 4 to 8 lowers the viability 52.5 percent. From point 8 toward the left the curve sharply flattens out and parallels the base line. X-hyperploid females with right-hand duplications 9R and M5R are only slightly more viable than the triple-X females.

The analysis of these two sets of curves indicates that the effects of duplications on fertility and on viability are more or less independent of each other. This is shown by the fact that while the two fertility curves are

very similar and symmetrical, the two viability curves are dissimilar and asymmetrical. Furthermore, the left fertility curve intersects the left viability curve, and likewise the right fertility curve intersects the right viability curve. If the effects were closely correlated, we should expect these two sets of curves to parallel each other.

The effect on fertility of adding sections of equal length from either end of the active region of the chromosome is about the same. Thus, on PAINTER'S cytological map, the ratio of duplication w13L to that of 8R is as 134 is to 137, and w13L females show a percentage of fertility of 22.3 as against a percentage of 26.7 for 8R females. These percents are clearly of the same order of magnitude. The difference is probably due to the fact that the 8R duplication is slightly longer than that of w13L, and, in addition, contains the inert region, which has no detrimental effect on fertility. Furthermore, the number of females with the 8R duplication available for testing was only about one-fifth as large as that for w13L. Hence, the determined percent of 26.7 for the 8R class is more subject to error.

These results indicate that the genes affecting fertility are relatively numerous, of the same degree of strength, and are uniformly distributed along the X chromosome, with the exception of the genes in segment 13R. The genes in this segment affecting fertility are either balanced or absent. These suggestions are based upon the study of relatively long segments. It is possible that a study of shorter segments might show regional variations. We have reached these conclusions by the following calculations, which are shown in table 3.

TABLE 3

LEFT DUPLICATIONS				RIGHT DUPLICATIONS			
STOCK	LENGTH IN MM	CALCULATED FERTILITY PERCENTS	ACTUAL FERTILITY PERCENTS	STOCK	LENGTH IN MM	CALCULATED FERTILITY PERCENTS	ACTUAL FERTILITY PERCENTS
M5	36	79.5	84.6	13	0	100	102.2
9	72	59.0	66.6	1	36	74.8	79.5
w13	134	23.6	22.3	4	59	58.7	69.3
8	158	9.9	7.8	8	106	25.8	26.7
S	175	0	..	w13	130	9.0	10.6
4	205	0	0	S	143	0	0
1	227	0	0	9	192	0	0
13	264	0	0	M5	228	0	0

The theoretical minimum length which produces sterility was taken as an average of the lengths of the segments bounded by the ends of the chromosome and the points where the projections of the last segments of left and right fertility curves cut the base line. This length was found to be 175 mm on the salivary map. Figure 15 shows that the 32 mm representing

duplication 13R has but little influence on fertility. Hence, 32 mm have been subtracted from all right-hand duplication lengths, including the theoretical minimum sterility length. The expected drop in fertility has been calculated from the ratio of length of the segment involved to the minimum sterility length. The close correspondence between the actual and calculated percentages render these assumptions probable.

The effect on viability of adding sections of approximately equal length from either end is strikingly different. Thus the percentage of viability of M5L females, with a duplicating piece of 36 mm, is 47.8, while 13R females, with a duplicating piece of 32 mm, show a percentage of viability of 192.7. The 9L females, with a duplication of 72 mm, give a viability percentage of 43.7, but 1R females have a viability percentage of 129.3 with a duplication length of 68 mm. Finally, w13L females, with a duplication of 134 mm, give a percentage of 42.3, while 8R females show a percentage of only 4.1, although the duplication is 137 mm long.

These comparisons of sections from opposite ends of the chromosome are sufficient to emphasize the point that with respect to their effect on viability, duplications from different regions of the chromosome are qualitatively different. This is not true of their effect on fertility. With the exception of 13R, duplications of equal length from different regions have about the same effect on fertility. The degree of sterility produced by different duplication X-hyperploid females is probably proportional to their actual cytological lengths. It may be that a more detailed study will show regional differences of a minor character. In its implications on sexual balance, this conclusion is similar to the one reached by DOBZHANSKY and SCHULTZ (1934) as a result of their study on the effects of duplications on the intersexes from triploids. It is to be expected that the effects of duplications would be different on two such vital phenomena as those of fertility and viability. The fundamental difference between the two sexes is not that of viability, but rather it is the ability to produce either male or female gametes. Fertility is therefore a much more critical measure of this function than viability and should be more closely linked with sexual balance.

As we have shown, the genes influencing fertility of the hyperploids are distributed along the X chromosome. They probably constitute the female-tendency genes of BRIDGES. One of the principal effects of adding them in larger and larger doses, through duplications, is to progressively lower fertility, finally culminating in sterility. These genes in triplicate obviously upset the normal balance.

Part of the expenses of this investigation was met by a grant to the senior author from the Committee on Radiation of the NATIONAL RESEARCH COUNCIL.

SUMMARY

1. A study was made of the effects on the phenotypic expression, viability, and fertility of duplicating pieces of various lengths of the X chromosome in hyperploid females.

2. The phenotype of the hyperploid in general depends on the section of the chromosome in duplication.

3. The viability of the hyperploid also depends on the section duplicated.

4. The fertility of the hyperploid is inversely proportional to the length of the duplicating piece, with the exception of the carnation to spindle-fiber end interval, where the genes influencing fertility are either in balance or else are absent.

LITERATURE CITED

DOBZHANSKY, TH. and SCHULTZ, JACK, 1934 The distribution of sex factors in the X chromosome of *Drosophila melanogaster*. J. Genet. **28**: 349–386.

PAINTER, THEOPHILUS S., 1934 The morphology of the X chromosome in salivary glands of *Drosophila melanogaster* and a new type of chromosome map for this element. Genetics **19**: 448–469.

PATTERSON, J. T., STONE, WILSON, BEDICHEK, SARAH, and SUCHE, META, 1934 The production of translocations in Drosophila. Amer. Nat. **68**: 359–369.

BIPARENTAL MALES AND HATCHABILITY OF EGGS IN HABROBRACON[1]

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ARTHUR M. CLOUDMAN (WHITING and ANDERSON 1932, p. 423) made a comparative study of the fecundity of mated and unmated females of *Habrobracon juglandis* (Ashmead). The mean number of offspring from the vials producing males and females was 13.69 while the vials producing only males averaged 18.05. The difference, 4.39 ± 1.30,[2] is in favor of the unisexual fraternities. Thus some factor arising in connection with fertilization reduced the fecundity of mated females.

ANNA R. WHITING (WHITING and ANDERSON 1932, p. 425) made a series of observations on the fecundity of several kinds of females. Counts were made showing the number of eggs laid, the hatchability of the eggs, and the viability of the larvae. Females of various types produced approximately the same average number of eggs per day. Hatchability, larvae/eggs, and viability, adults/larvae, were each about 80 percent for females set as virgins or mated to unrelated males, while hatchability was greatly lowered (49.1 percent) and viability somewhat decreased when females were crossed to related males. This suggested that fertilization of eggs by related sperm renders some eggs incapable of developing very far. Since biparental males are known to be produced in crosses involving related stocks, where they can be distinguished from their azygotic brothers, the question as to a possible relation between the occurrence of biparental males and fecundity suggested itself.

The investigations reported at this time were undertaken in order (1) to determine the comparative fecundity of females bred as virgins or mated to males from one or the other of three different stocks; (2) to compare the hatchability of eggs and viability of later stages from virgins and females mated to the various kinds of males; (3) to determine what relation, if any, exists between fecundity and the occurrence of biparental males; and (4) to find any possible evidence to support the idea expressed by other investigators that biparental males are produced in a homozygous stock where they cannot be distinguished from azygotic males by their characteristics.

Acknowledgment should be made to the Committee on Effects of Radiation on Living Organisms (NATIONAL RESEARCH COUNCIL) who through

[1] Contribution from the Department of Zoology, and published with the approval of the Director of the NORTH CAROLINA EXPERIMENT STATION as Paper No. 78 of the Journal Series.

[2] Standard errors are used throughout this paper.

grants to Dr. P. W. WHITING have furnished technical assistance toward the completion of this work. Grateful acknowledgment is also made to Dr. P. W. WHITING for advice and criticism.

MATERIALS AND METHODS

Females used in all crosses belonged to stock 11-o, an orange-eyed stock derived by grading orange (from stock 3, Lancaster, Pennsylvania) up to wild-type stock 11 (Iowa City, Iowa). This stock was chosen because stock 11-o females crossed to stock 11 males produce a larger percentage of biparental males than any other stocks available. The females were divided into four groups: some were bred without being mated, some were mated to males of stock 11-o, some to males of stock 11, and others to unrelated males of wild-type stock 25 (New York City). Actual mating of the female and male was observed in each case, but two of the females mated to males of stock 11-o produced only male offspring. In the summary these females are grouped with those set without mating.

Females were set individually in small tin boxes, each lined with a piece of paper cut and fitted carefully, and containing a caterpillar which had already been paralyzed by another wasp. Each caterpillar was examined under a binocular to remove any eggs which might have been laid by the female which stung it. By using paralyzed caterpillars immobility was insured, giving the best conditions for egg laying and for subsequent examination. Observations were made daily on the contents of each tin box and the females were transferred to new boxes. Records were kept each day of the number of eggs laid by each female, the number of eggs that developed into larvae, and the number of larvae that reached the pupal stage. If it became apparent that one host was not going to be sufficient food for all the larvae on it, another paralyzed caterpillar was added to the box. When adults began to emerge, daily counts of the offspring were made. The mothers were transferred for an average of about thirteen days each. None was set after fifteen days because previous experience had shown that females usually exhaust their supply of sperm after fifteen or sixteen days and breed thereafter as virgins. Cultures were kept at 30° C except when observations were being made.

DATA AND DISCUSSION

In table 1 eggs/days represents egg production per female per day; larvae/eggs represents hatchability of eggs; pupae/larvae gives the percentage of pupation; and adults/pupae expresses the percentage of eclosion.

Virgin females. Summaries of data are given in table 1. All the offspring were males as expected.

Matings of unrelated stocks (stock 11-o females by stock 25 males). Hatch-

TABLE 1

STOCK 11-o FEMALES	NO. SET	TOTAL DAYS SET	EGGS	LARVAE	PUPAE	ADULTS ♂♂	ADULTS ♀♀	EGGS DAYS	LARVAE ×100 EGGS	PUPAE ×100 LARVAE	ADULTS ×100 PUPAE	BIPARENTALS ×100 TOTAL
Unmated	9	119	2501	1979	1921	1733		21.02	79.1 ± .81	97.1 ± .37	90.2 ± .68	
Mated to stock 25 males	8	109	2289	1905	1868	452	1306	21.00	83.2 ± .78	98.0 ± .32	92.5 ± .61	74.3 ± 1.05
Mated to stock 11 males	6	80	1682	1179	1086	393 140*	436	21.03	70.1 ± 1.1	92.1 ± .78	89.2 ± .94	59.4 ± 1.6
Mated to stock 11-o males	7	99	2022	1351	1245	560	523	20.42	66.8 ± 1.05	92.2 ± .73	87.0 ± .95	48.3 ± 1.5

* Biparental males, 24.3 ± 1.8 percent of total biparentals.

ability of the eggs and percentage of pupation were about the same as for virgin females. Of the offspring, orange-eyed males and black-eyed females, 74.3 ± 1.05 percent were females, indicating that the majority of the eggs were fertilized.

Matings of related stocks (*stock 11-o females by stock 11 males*). The offspring were orange-eyed males (azygotic), black-eyed males (biparental), and black-eyed females. Of the offspring 59.4 ± 1.58 percent were biparentals, and 24.3 ± 1.79 percent of the biparentals were males. A significantly lower proportion of the offspring were biparentals than from the cross to males of stock 25. Hatchability was also significantly less.

Matings within one stock (*11-o females by 11-o males*). The offspring consisted of orange-eyed males and females. Since the parents were similar in eye-color, biparental males, if present among the offspring, could not be distinguished by inspection from their azygotic brothers. The females laid practically the same number of eggs per day as the females in each of the other experiments. Hatchability was somewhat lower and there were fewer distinguishable biparentals than from other matings.

Presence and type of sperm failed to affect the egg productivity since females of all four groups laid approximately the same number of eggs per day. Any differences among the groups are due then to fertilization. Virgin females and those crossed to unrelated males of stock 25 give similar results, in that about the same percentage of eggs developed into larvae. From females mated to related males of stock 11 there is a much lower hatchability of eggs. Among the adults from this cross were biparental

males. It is very interesting that associated with lowered hatchability of eggs and lessened viability of later stages there is the production of biparental males, while associated with higher hatchability and viability there is an absence of biparental males. Thus it seems that whatever mechanism causes the production of biparental males simultaneously renders a considerable portion of the eggs and later stages incapable of further development. This suggests that the production of biparental males may be either due to or followed by some abnormal arrangement of the chromosomes, which prevents the development of certain individuals, and strongly supports WHITING'S (1933) explanation of the mode of sex determination and the occurrence of biparental males.

The crosses within the stock (to 11-o males) gave results strikingly similar to those from the cross to related males of stock 11. In table 1 it may be seen that for egg hatchability the two crosses are similar, in that the values for each are significantly different from those from the offspring of virgin females or females mated to unrelated stock 25. The percentages of eggs becoming adults are for the stock 11-o and stock 11 crosses 53.6 ± 1.1 and 57.6 ± 1.2, respectively, while for the outcross the value is $76.8 \pm .88$. Further comparison shows for the stock 11-o and stock 11 crosses biparental ratios of 48.3 ± 1.52 and 59.4 ± 1.58, respectively. The corresponding value for the outcross is 74.3 ± 1.05. All these values are significantly different.

Comparison of these figures leads one to believe that although biparental males cannot be detected, they are being produced, because the low hatchability of eggs and poor viability of later stages are very similar to that shown by the cross where biparental males are detectable. Since egg hatchability is 3.3 ± 1.5 percent lower than in the cross to stock 11 males, it suggests that even more biparental males are being produced. The lower ratio of distinguishable biparentals in this cross is due then to the inclusion of biparental males with the azygotic males.

Further evidence that biparental males are present among the offspring of the females crossed to like males was obtained by making fertility tests. It has been shown by several investigators that a large proportion of biparental males is sterile, and that even those which show some fertility cause the production of very few daughters. Seventy of the male offspring from stock 11-o by stock 11-o were selected at random and mated to virgin females. All the matings were carefully observed. Eighteen of the females produced only male offspring, which indicated that the males which were mated to these females were sterile. The smallest number of males produced by any female was 12, while the average was 30. Seven matings produced male and female offspring as follows, males being given first: 19, 2; 27, 1; 22, 3; 35, 1; 29, 2; 15, 1; 30, 2. The males used in these matings

were probably biparental also, as judged by the small number of daughters produced. Forty-five of the matings produced males and females in large numbers, the mean number being 6.7 males and 26 females. There is ample justification for the conclusion that the sterility of the majority of the twenty-five males, or about 36 percent of those tested, was due to their biparental constitution.

It should be noted that about the same proportion of larvae became pupae among the offspring of the virgin females and those mated to unrelated males of stock 25. On the other hand, significantly fewer larvae became pupae in the crosses to related stocks 11 and 11-o. Excess pupation among offspring from virgin females over offspring of crosses to stocks 11 and 11-o was $4.97 \pm .87$ percent and $4.92 \pm .83$ percent, respectively. Excess pupation of the outcross was $5.87 \pm .84$ and $5.82 \pm .80$, respectively. Similarly fewer pupae became adults in the crosses to stocks 11 and 11-o.

Eggs and pupae were less viable from the cross to stock 11-o males, showing that a greater proportion of individuals is rendered incapable of development than in any other cross. In the light of this and other evidence, the conclusion is fully justified that biparental males were produced in this cross. This conclusion also suggests that other inbred stocks in Habrobracon produce biparental males which are not recognizable.

DZIERZON'S LAW

DZIERZON many years ago advanced the theory that all the eggs produced in the ovary of queen bees are alike; those developing without fertilization become males, and those fertilized become females. Because not all the eggs of an unfertilized queen hatch, some have believed DZIERZON'S theory to be partly incorrect. One sentence will be quoted from PHILLIPS' text of Beekeeping (1922): "It seems clear, however, that the statement of DZIERZON that all the eggs in the ovary are male eggs cannot be accepted and it is, in fact, not improbable that the eggs destined to be females die for want of fertilization, while the eggs destined to be males, not requiring fertilization, are capable of development." Somewhat the same idea is expressed in the revised edition of PHILLIPS' text (1928): "The author has found that many eggs laid by drone-laying queens fail to hatch and, in fact, are often removed in a short time by the workers. This makes it impossible to accept DZIERZON'S statement that all eggs laid by such a queen become males and the statement must be modified as follows: all of those eggs laid by a drone-laying queen which develop become males. The potentialities of the eggs which never hatch are not known."

For the first time, as far as is known to the author, actual egg counts for mated and unmated females have been made for an insect probably com-

parable to the honey bee. There can be no doubt that in Habrobracon all the eggs of a virgin are alike for sex. Nine unmated females laid 2,501 eggs, of which 79.1 ± .81 percent hatched into larvae. Of the eggs 69.3 ± .92 percent were able to develop into adults, all males. Eight females, mated to unrelated males, laid 2289 eggs, of which 83.2 ± .78 percent became larvae and 76.8 ± .88 adults. Of the adults, 74.3 ± 1.05 percent were females. If the 21.9 percent of the eggs from unmated females failed to hatch because they were destined to be females, an unusual mechanism for the segregation of eggs for sex has occurred to give such a ratio. In addition, if the 21.9 percent failed to hatch because they were destined to be females, why should 57.1 percent of the eggs from mated females hatch and develop into females? Recent investigations have served to corroborate the views of DZIERZON, adding to his beliefs the knowledge that occasionally males are biparental.

SUMMARY AND CONCLUSIONS

1. Females of stock 11-o lay the same number of eggs whether bred as virgins or mated to males of the same stock, of a related stock, or of a totally unrelated stock.

2. Hatchability of eggs is high from virgins and from matings involving unrelated stocks. Hatchability of eggs is lower from crosses of related stocks, in which biparental males are known to be produced and within a stock in which the evidence indicates they are produced.

3. The percentages of hatchability of eggs, pupation of larvae, and eclosion of pupae are very nearly the same in the crosses to males of related stocks 11 and 11-o, although lower in the latter. Thus it seems likely that biparental males are being produced in the latter cross, where they cannot be distinguished in appearance from their uniparental brothers, as well as in the former cross, where they are distinguishable.

4. Further evidence for the production of biparental males by inbreeding of stock 11-o was shown by the sterility of about thirty-six percent of the male offspring tested.

5. Although the causes underlying the production of biparental males are not known, it may be of considerable value to know that associated with the appearance of such males is a decreased hatchability of eggs and viability of offspring.

6. All the eggs produced by a virgin female are equivalent for sex.

LITERATURE CITED

PHILLIPS, E. F., 1922 Beekeeping. p. 188. The Macmillan Company.
1928 Beekeeping. p. 200. The Macmillan Company.
WHITING, P. W., 1933 Sex-determination in Hymenoptera. Biol. Bull. **65:** 357.
WHITING, P. W. and ANDERSON, R. L., 1932 Temperature and other factors concerned in male biparentalism in Habrobracon. Amer. Nat. **66:** 420–432.

GENETICAL STUDIES OF MONOSOMIC TYPES OF NICOTIANA TABACUM

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INTRODUCTION

In organisms having two sets of chromosomes that pair normally, bivalents being regularly formed at the first metaphase of meiosis, it is sometimes possible to obtain types that lack a chromosome, yet exhibit no other marked irregularities in chromosomal behavior. Such individuals are monosomics (2n-1) and, as a rule, are abnormal only in the sense that some specific chromosome is without a homolog.

Since monosomism destroys the original duality of one chromosome pair, an unbalanced genetical system is set up, capable of variation, as reflected both in the immediate individual and its progeny. Reports of types derived apparently from monosomic individuals, in many cases markedly deviating from normal, are prevalent in the literature of polyploid plants. Such variants, many of which have higher or lower chromosome numbers, fragments, or structural chromosomal rearrangements, are mentioned in numerous papers (see especially NISHIYAMA 1931, 1933; R. E. CLAUSEN 1930, 1931; POWERS 1932; HAKANSSON 1932). Few studies, however, have been exclusively devoted to the genetics and cytology of monosomic forms. Obviously processes that may modify chromosome number and structure are not without practical importance, since they may serve as a means of inducing variation and obtaining improved types of cultivated plants.

In common with many other polyploid plant genera, monosomic types have arisen spontaneously in Nicotiana. AVERY (1929) reports their occurrence in the 9-paired species *N. alata* var. *grandiflora*. In *N. tabacum* var. *purpurea* a form known as "fluted" or haplo-F has arisen spontaneously in cultures in the frequency of about 1:150 (CLAUSEN and GOODSPEED 1926a). Monosomic types have been isolated in designed experiments by backcrossing F_1 hybrids to one of the parental types with lower chromosome number. In this way CLAUSEN and GOODSPEED (1926b) obtained the derivative "corrugated" from the *sylvestris-tabacum* hybrid. LAMMERTS (1932), working with the allied species *N. rustica*, used the same method in isolating seven different monosomic types. GOODSPEED and AVERY (1933) have recovered monosomics in progeny from X-rayed Nicotiana.

MATERIAL AND METHODS

The seven monosomic (2n-1) types dealt with in this paper are those of *Nicotiana tabacum* var. *purpurea*. This particular variety of tobacco is a highly homozygous strain, having been inbred continuously for over 27 years at the UNIVERSITY OF CALIFORNIA.

According to the theory advanced by GOODSPEED and CLAUSEN (1928), the polyploid species *N. tabacum* (2n = 48) has arisen by interspecific hybridization between two twelve-paired species, followed by a doubling of the chromosome number (amphidiploidy).

With the exception of haplo-F and haplo-C, of whose origin we shall speak in a later paragraph, all the monosomics have been obtained by interspecific hybridization and repeated backcrossing of selected F_2 derivatives to the normal *purpurea* variety. Hybrids are readily obtained between *tabacum* and *sylvestris* or *tomentosa*. The F_1 hybrids *sylvestris-tabacum* and *tomentosa-tabacum* are completely self-sterile when their own pollen is applied and typically show $12_{II}+12_{I}$ at first metaphase.

Seed of either the *sylvestris-tabacum* or *tomentosa-tabacum* hybrid may be obtained by backcrossing to *tabacum*. The progeny grown from seed of the *sylvestris-tabacum* backcross comprises a highly diversified collection of derivatives, which may be grouped into three general classes: (1) almost completely sterile, aberrant forms; (2) plants which resemble *tabacum*, but which are also highly sterile and give no progeny on self-fertilization; (3) *tabacum*-like forms that are somewhat fertile and produce in the course of a few generations plants identical with the original *tabacum* parent (GOODSPEED and CLAUSEN 1922). By cytological examination of the more fertile types, some are found to be monosomics. The process of backcrossing the partially fertile derivatives having a number of univalents to *tabacum* can be continued until other monosomics are isolated in succeeding generations.

By the method described above, it is theoretically possible to obtain twelve different monosomics of *tabacum*, types lacking one of the twelve *tabacum* chromosomes homologous with those of *tomentosa*. For convenience and distinction, these twelve haplo-types are to be designated A to L and may be appropriately called the "*tomentosa*" monosomics of *tabacum*. Four of these, A, B, C, and F, are considered in the present study.

In like manner, by backcrossing the F_1 *tomentosa-tabacum* hybrid, it is theoretically possible to obtain another series of twelve *tabacum* monosomics, in which the monosome is homologous with one pair of the chromosomes constituting the species *sylvestris*. This series is to be labeled M to X and constitutes the "*sylvestris*" monosomics of *tabacum*. Of these, haplos N, Q, and R have been selected for study.

Haplo-C has been the subject of a previous paper by CLAUSEN and GOODSPEED (1926b). Its lineage dates from 1915, when an F_1 *macrophylla-sylvestris* hybrid was backcrossed to the normal *macrophylla* parent. *Macrophylla* is a broadleaved, dwarf variety of the *tabacum* species. Several years later this monosomic of *N. tabacum* var. *macrophylla* was backcrossed a number of times to the *purpurea* variety, and the monosomic of the latter was isolated. This is the monosomic C dealt with in the present study.

Haplo-F and its derivatives have been more extensively investigated than any other monosomic type of *tabacum*. It is the F chromosome that carries the coral and mammoth complexes (CLAUSEN 1931). Because of the very characteristic waviness of the flower limb, this type has been described as "fluted" (CLAUSEN and GOODSPEED 1926a). The mechanism responsible for the occurrence of "fluted" has been attributed to non-conjunction of the F chromosome. Thus CLAUSEN (1931) has observed that occasionally at first metaphase there are $23_{II}+2_I$ instead of the normal 24_{II}.

Because of its mode of origin, haplo-F was from the time of its inception a genetically homogeneous type. This was not the case with the other monosomics, which, as already noted, were isolated by interspecific hybridization and selection. The facility with which monosomics may be obtained by this method is somewhat lessened because, once isolated, they must be backcrossed a number of times to the normal *purpurea* so as to reduce the quantity of genetic material contributed by the alien species to a minimum.

MORPHOLOGY OF THE MONOSOMIC TYPES

Since the effect of chromosomal removal is a complex one, disturbing the genetic balance in its entirety, the morphological changes exhibited by these types are largely the result of genic deficiencies, which alter the interrelationships and factor balance previously associated with a normal phenotype. The removal of any one chromosome in *N. tabacum* var. *purpurea* produces rather general morphological changes. The flowers of the seven haplo forms are pictured in figure 1. Some of the features which most markedly distinguish each monosomic type from the normal strain are as follows:

Haplo-A: Reflexed corolla limb. Anthers are exserted.

Haplo-B: The leaf auricles are attenuated and narrow. The corolla limb is flat and intensified crimson in color.

Haplo-C: Large pendant flowers, the throats of which are light pink.

Haplo-F: The expanded flower limb is undulated and the entire inflorescence telescoped.

Haplo-N: Similar to haplo-F, but the flower limb is not fluted.

Haplo-Q: Plant of slender growth habit. The flower has a narrow throat and small limb.

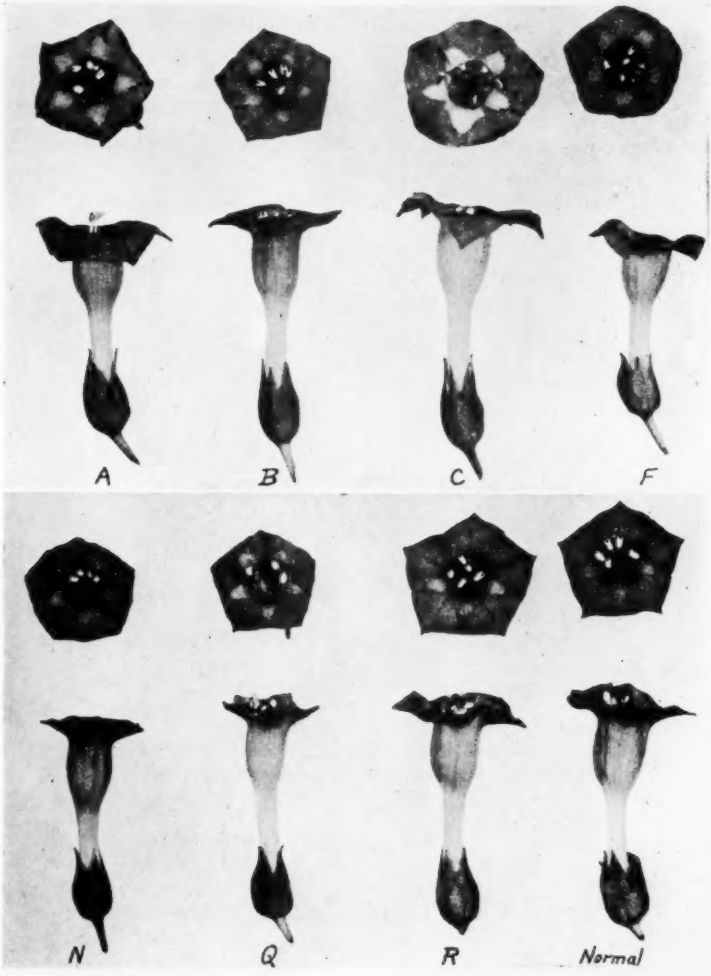

FIGURE 1.—Typical flowers of the monosomic types and the normal *purpurea* strain.

Haplo-R: Flower has a very broad limb and is coral pink in color. The leaves are corrugated.

Seed Production

The chances of a monosomic individual's establishing itself under natural conditions and successfully competing with the normal phenotype would partly depend upon its fertility. The normal strain of *purpurea* yields approximately 4000 seeds per capsule. Every monosomic type investigated produces a small amount of seed in comparison with the normal strain. The average number of seeds from typical capsules of haplos A, F, and N is, by actual count, less than one-sixth of the number maturing in the normal *purpurea*. Even the types comparing most favorably—namely C, Q, and R—produce only about one-third of the normal seed yield.

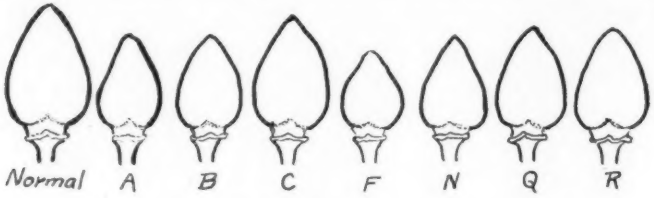

FIGURE 2.—Capsules of the seven monosomic types and the normal strain of *N. tabacum* var. *purpurea*.

The reduced seed yields are perhaps largely explainable on the basis that the capsules are merely reduced in size, in common with other morphological changes accompanying monosomism (figure 2). However, there are indications that a large number of ovules or young zygotes must abort before reaching maturity.

Pollen Production and Morphology

Striking are the differences existing between the pollen grains of most of the haplo types when viewed microscopically. The pollen of each monosomic type, with the possible exception of haplo-A, is dimorphic. Two general classes of pollen may be distinguished, one consisting of large and apparently morphologically normal grains, the other composing a majority of the total number, which are considerably smaller and have characteristic densely granular deposits within them (figure 3). In addition there is a small and variable number of aborted grains, appearing shriveled and almost devoid of plasma.

TRANSMISSION OF THE MONOSOMIC CONDITION

Progenies from the cross monosomic ♀ × normal ♂ and from the reciprocal combination were grown at the University Farm, Davis, and also at the UNIVERSITY OF CALIFORNIA, Berkeley, during the summer of 1933 for the purpose of obtaining the transmission ratios of the monosomic

condition through egg and pollen respectively. From four to six populations, consisting of approximately 35 to 70 plants each, were grown from a single cross.

FIGURE 3.—Pollen of haplo-N. The smaller grains with the granular deposits are assumed to be nullosomic for the N chromosome. Mounted in xylol-balsam. ×300.

Through the Egg

The summarized data presented in table 1 show that the number of monosomic plants obtained in the progeny largely depends upon the particular chromosome that has been removed from the genom. Since the number of plants dealt with forms a reasonably large random sample, the differences existing in the transmission ratios are undoubtedly significant, especially since the results obtained for each population of a given type were fairly consistent.

Of the seven types, haplos A, Q, and R are outstanding in their high transmission of the monosomic condition. In haplo-A, 83.2 percent of the

TABLE 1

Transmission of the monosomic condition; monosomic as female parent.

MONOSOMIC SERIES	TYPE	NORMAL	MONOSOMIC	TOTAL	PERCENT* MONOSOMIC
"tomentosa"	A	40	243	292	83.2
	B	117	160	284	56.3
	C	62	153	226	67.7
	F	92	162	264	61.4
"sylvestris"	N	112	99	217	45.6
	Q	48	209	262	79.8
	R	41	175	223	78.5

* A small percentage of the total number were plants classified as "aberrant."

progeny are plants having only 47 chromosomes; in Q 79.8 percent; and in R 78.5 percent. The highest transmission reported in *N. rustica* (LAMMERTS 1932) was 75 percent. Five of the seven monosomics for which he reported transmission ratios showed a percentage varying from 56 to 75 for the number of functional (n-1) gametes, assuming that all or practically all the seed germinated and eventually produced mature plants.

Theoretically, one might expect gametes having 23 and 24 chromosomes in equal numbers. With the single exception of haplo-N, all the types produce a preponderance of 23-chromosome gametes. In general, this phenomenon has been attributed by various workers to the failure of the univalent chromosome to be regularly incorporated in the daughter nuclei during megasporogenesis. Differential fertility and survival are factors that cannot be neglected.

That the viability of monosomic zygotes may be somewhat less than normal is suggested by the seed germination data. The average number of seeds germinating in the cross monosomic ♀ × normal ♂ is about 84 percent—10 percent lower than when the seed from a normal plant pollinated with monosomic pollen is sown. Such a result might be expected if those zygotes having an unbalanced chromosome number are less viable than zygotes having all 48 chromosomes. Monosomics of *purpurea* also seem to be slightly less vigorous than their normal sibs. That they are usually retarded in time of development is borne out by the successful practice of selecting the later-developing seedlings of a progeny, so that the monosomic type may be perpetuated by growing only a small number of plants. Whereas haplos Q and R, for instance, ordinarily produce about 78 or 79 percent of monosomic offspring, this percentage may be increased to 100 if only the seedling plants that are somewhat delayed in growth are selected for preservation. Moreover, it is among such groups of retarded individuals that most of the plants classified as "aberrant" have

been discovered. There is reason to believe, therefore, that the actual number of functional (n-1) gametes is much higher than that found indirectly by counting the number of monosomic plants, because of a decreased adaptability to environmental conditions brought about by the change in genic balance. "Functional" gametes are herewith defined as those producing mature seeds.

LAMMERTS (1932) suggested that the differences which he noted in some of the female transmission ratios of different monosomic types in the theoretically amphidiploid species *N. rustica* might be attributable to a differential lagging of the monosome, so that some univalents were more liable to be eliminated than others.

It should therefore be possible to demonstrate here, for example, that elimination of the unpaired chromosome is much more frequent in such a type as haplo-A, which gives approximately 83 percent of functional (n-1) gametes, than in a type like haplo-B, where about 56 percent of (n-1) eggs are functional, if relative viability of the seed is neglected.

As a measure of the frequency of univalent elimination during microsporogenesis, the number of microcytes or micronuclei can be counted in the tetrad stage, and the approximate number of n and (n-1) gametes calculated (NISHIYAMA 1931). By employing iron aceto-carmine smears, one can readily observe the number of microcytes and micronuclei. In table 2 each group of observations pertains to the results obtained by smearing anthers from one flower. Usually counts were made of from 150 to 200 tetrads from a single slide. In haplos A and B the slides were prepared from material collected over a period as long as two months, although adherence to any collection schedule was neglected. The results vary considerably from slide to slide.

On comparing the averages of the number of microspores calculated to be deficient for a monosome, one sees that the variation between the different monosomic types is insignificant and incapable of explaining the large excess of monosomic plants actually obtained in some haplo types. Thus as shown by table 3, haplo-A produces over 83 percent of monosomic progeny, whereas the calculated number is about 77 percent. From the previous considerations on the relative viability and survival of the types with unbalanced chromosome number, this discrepancy would be even larger.

For the moment, if we make allowance for the lower germination percentages obtained in the cross monosomic ♀ × normal ♂, the transmission ratios through the egg would be converted as shown in table 3. The differences in germination percentages reported are assumed to have resulted solely from the mortality of (2n-1) zygotes. The average decrease in seed viability in column 2 was obtained by subtracting the observed germina-

TABLE 2

Frequency of univalent elimination in various monosomic plants.

MONOSOMIC TYPE	PLANT NUMBER	MICROCYTES AND MICRONUCLEI PER TETRAD				RATIO (n-1):n	PERCENT (n-1)
		0	1	2	2+*		
A	34240P1	26	55	35	0		
		47	93	92	0		
	34240P2	65	45	89	4		
		51	27	69	3		
		41	22	25	2		
		39	15	37	1		
		53	22	37	1		
		322	279	394	11	3057:923	76.8
B	34244P7	63	43	75	7		
		49	25	47	2		
		41	30	54	4		
		37	12	35	3		
		36	16	29	0		
		226	126	240	16	1790:578	75.6
N	34256P2	46	32	56	8		
		79	56	94	13		
		125	88	150	21	1114:338	76.7
R	34264P6	35	25	45	3		
	34264P8	49	28	41	0		
		52	40	51	2		
		44	28	44	3		
		180	121	181	8	1447:481	75.0

* Tetrads containing more than two microcytes or micronuclei and omitted from the calculations in determining ratios and percentages of (n−1) gametes.

tion percentage of each haplo type from the average percentage of the reciprocal crosses. The data presented in table 3, though limited, indicate that differential lagging cannot account for such excesses of monosomic plants.

We shall now consider the exceptional instance of egg transmission in which the number of functional (n−1) gametes was below the expected value of 50 percent, as determined by counting the number of monosomic plants in the progeny. Again according to table 1, one type, haplo-N, produced 45.6 percent of monosomic plants—considerably below that obtained for any other type. But, as shown in table 3, this fact may well be explained by the selective elimination of (2n−1) zygotes, which fail to germinate. This appears all the more probable if we compare the germina-

TABLE 3

Corrected transmission ratios of the monosomic types compared with the expected percentages, as calculated from the frequency of univalent elimination during microsporogenesis.

MONOSOMIC TYPE	AVERAGE DECREASE IN SEED VIABILITY	PERCENTAGE OF MONOSOMIC PROGENY (TABLE 1)	CORRECTED PERCENTAGE OF (2n−1) PLANTS	CALCULATED PERCENTAGE (TABLE 2)
A	4.2	83.2	87.4	76.8
B	20.1	56.3	76.4	75.6
C	3.5	67.7	71.2	..
F	6.6	61.4	68.0	..
N	25.1	45.6	70.7	76.7
Q	2.5	79.8	82.3	..
R	5.2	78.5	83.7	75.0

tion percentages with the number of monosomic plants surviving. Thus the populations of haplo-B and haplo-N, which produce the smallest proportion of monosomic plants, also manifest the lowest seed viability. If this is a logical explanation, the number of (2n−1) zygotes in haplo-N is approximately 71 percent. Seed viability and ultimate survival thus appear to be important factors in interpreting the transmission ratios, and one is led to conclude that all the monosomics of *tabacum* discussed herein produce an excess of (n−1) egg cells.

Through the pollen

Only a very small percentage of (n−1) pollen grains succeed in fertilizing the ovules of normal plants. The tabulated results are collected in table 4. The highest number of monosomic plants was obtained in the case of haplo-F, where over 7 percent of the progeny were 47 chromosome plants. Haplo-A, which has been shown to transmit the monosomic condition so readily through ovules, produces only a few monosomic plants when used as male parent.

TABLE 4

Transmission of the monosomic condition, normal ♀ × monosomic ♂.

MONOSOMIC TYPE	POPULATION NUMBER	NORMAL	MONOSOMIC	ABERRANT*	TOTAL NUMBER PLANTS
A	33208	47	2		49
	33209	45	4	1	50
	33210	48	3		51
	33211	43	1		44
	Totals	183	10	1	194
	Percent	94.3	5.2	0.5	
B	33212	49	1	1	51
	33213	41	2	2	45
	33214	41	1		42
	33215	40	2	1	43

TABLE 4 (*Continued*)

Transmission of the monosomic condition, normal ♀ × monosomic ♂.

MONOSOMIC TYPE	POPULATION NUMBER	NORMAL	MONOSOMIC	ABERRANT*	TOTAL NUMBER PLANTS
	Totals	171	6	4	181
	Percent	94.5	3.3	2.2	
	33216	44			44
C	33217	45		1	46
	33218	48			48
	33219	43			43
	Totals	180		1	181
	Percent	99.4	0.0	0.6	
	33220	39	5	3	47
	33221	40	4	3	47
F	33222	27	3		30
	33222	11	1	3	15
	33223	51	1		52
	Totals	168	14	9	191
	Percent	88.0	7.3	4.7	
	33224	51	2		53
N	33225	55		2	57
	33226	52	4	1	57
	33227	46		4	50
	Totals	204	6	7	217
	Percent	94.0	2.8	3.2	
	33228	52			52
Q	33229	51		1	52
	33230	49	1	1	51
	33231	45		2	47
	Totals	197	1	4	202
	Percent	97.5	0.5	2.0	
	33232	53	1	1	55
	33233	44	2		46
R	33234	48			48
	33234	1		1	2
	33235	46		1	47
	33235	2			2
	Totals	194	3	3	200
	Percent	97.0	1.5	1.5	

* The occurrence and cytology of aberrant plants in offspring of these monosomic types are discussed by the author in a companion paper (1935).

There is a large variability in the number of monosomic plants in different populations of the same cross. According to table 4, population 33220 segregated 5 monosomic plants out of a total of 47, whereas in population 33223, grown from the same seed lot, only one plant of the 52

was monosomic. The average differences between populations of any two monosomic types may be less significant than they appear. It seems probable, however, that, as in ovule transmission, certain differences are traceable only to the degree of unbalance caused by the absence of any specific chromosome.

A very low transmission of the monosomic condition through the pollen is characteristic of all the *purpurea* monosomics used in this study. This corroborates the findings of CLAUSEN and GOODSPEED (1926a), who reported only about 2 percent of functioning pollen grains of the 23 chromosome class in haplo-F. In the *macrophylla* derivative "haplo-C" the same authors (1926b) reported a pollen transmission of 3.3 percent, whereas in the present study the haplo-C *purpurea* was the only one of the seven types which failed to transmit the monosomic condition when used as male parent.

No correlation appears to exist between the transmission of monosomism through the ovules and pollen of the same haplo type. Thus haplo-F produced approximately 7 percent of monosomic plants as male parent, and 61 percent transmission was found through the ovules, whereas haplo-C failed to transmit through pollen yet gave rise to approximately 68 percent of monosomic plants when fertilized with normal pollen.

A large proportion of the functioning pollen grains must contain a chromosomal complement that has been the outcome of structural or numerical chromosomal rearrangements originating in the mother plant. The fact that such modified gametes are frequently pollen-transmitted accounts for the comparatively large number of off-type plants, which can be classified as neither monosomic nor normal (table 4).

DISCUSSION

Of the seven monosomic types reviewed in the present paper, each is readily distinguishable from the normal strain by a complex of characters, which are typically inherited as a unit. Each complex is general in nature, reflecting changes in the physiology of the whole plant rather than strictly localized ones.

The monosomic types of *N. tabacum* var. *purpurea* are somewhat retarded in development, besides being slightly less vigorous than the normal strain. The proportion of monosomic plants in a progeny may be increased by preserving only plants that are retarded in the seedling stage of development. LI (1927) showed that haplo-IV flies of *Drosophila melanogaster* were retarded in all three developmental stages and emerged two to three days later than normal sibs. AVERY (1929) described a monosomic form of *Nicotiana alata* var. *grandiflora* (2n = 18) as being less vigorous than the diploid, as is the case reported by KIHARA (1932) in *Pharbitis nil* (2n = 30).

In fatuoid oats (2n = 42), as illustrated by NISHIYAMA (1933), only a slight change occurs in the phenotypic appearance of plants monosomic for the *c* chromosome. POWERS (1932), working with some strains of cultivated wheats derived from interspecific crosses, stated that 41 chromosome plants were indistinguishable from the normal diploid. Obviously the character and extent of the underlying physiological changes may vary according to the genic content of the particular chromosome removed relative to the rest of the complement. Thus the removal of a chromosome in polysomic or polyploid forms has little effect, because of the compensation afforded by another chromosome or chromosomes performing the same or similar functions.

Characteristic of the monosomic types is a very high transmission of the monosomic condition via the egg. The percentage of (2n − 1) plants obtained in the progenies of haplo type ♀ × normal ♂ ranged from approximately 45.6 percent in haplo-N to as high as 83.2 percent in haplo-A. This range in variation is less pronounced if the viability of the seed is considered. If the decrease in seed viability is caused by the mortality of monosomic zygotes, then the number of functional (n − 1) eggs is always well above 50 percent (table 3). Yet even after such a correction, the difference between such types as haplo-A and haplo-F amounts to approximately 20 percent. One cannot logically state that the number of monosomic plants obtained in the progeny is an accurate measure of the number of (n − 1) egg cells originally produced. For this reason we have purposely defined "functional" egg cells as those which produce mature seed. At present, no data are available giving the approximate percentage of (n − 1) egg cells that are formed, and which become fertilized. NISHIYAMA (1928) determined the number of functional (n − 1) female gametes in two different monosomic spelt wheats ($20_{II}+f$) and ($20_{II}+g$) to be 71 and 72.7 percent respectively. This would indicate that the specific chromosome left in the univalent condition is not influential in determining the relative frequency in which (n − 1) egg cells are produced. In the latter case, however, the removal of a chromosome would not be expected to produce so profound a change in balance as occurs in *N. tabacum*, theoretically an amphidiploid.

If NISHIYAMA's (1928) conclusions in regard to univalent elimination in monosomic spelt wheats are applicable in the present instance, then the frequency of univalent elimination calculated from pollen mother cell tetrads should be approximately the same as the percentage of monosomic plants obtained in the progeny, after allowing for reduced viability. Of the four monosomic types thus analyzed, the percentage of (n − 1) microspores ranged from approximately 75 to 77 percent (table 2). Although admittedly the variability between individual counts is sometimes very large, the averages indicate that the frequency of univalent elimination

is practically the same, regardless of the specific chromosome left in the univalent condition. The haplo types such as N, C, and F, therefore, might show a lower ratio of transmission either because of differential fertility, or a differential production of (n−1) eggs. As yet we have no method of testing such an assumption. But any explanation for an excess of monosomic plants over the calculated number is not forthcoming, unless it be that univalent elimination occurs more frequently during megasporogenesis.

Whereas in monosomic hexaploid wheats and oats the results of KIHARA (1924), HUSKINS (1928), NISHIYAMA (1931, 1933), POWERS (1932), and others, demonstrate that 20-chromosome pollen is usually only at a slight disadvantage in competition with 21-chromosome pollen, the situation is much different in *N. tabacum* monosomics, where the highest number of (n−1) microspores effective in fertilization was approximately 7 percent in haplo-N. In haplo-C the monosomic condition was not pollen-transmitted. Judging from the frequency of univalent elimination during microsporogenesis, the ratio of (n−1) to n microspores is probably about the same in all of the types—namely, in the ratio of approximately 3:1. In 41 chromosome fatuoid oats, NISHIYAMA (1931) found the ratio to be 6:1. The same author (1928) reported a ratio of (n−1) to n egg cells in two 41 chromosome spelt wheats to be 3:1. KIHARA's (1924) results with emmer-spelt derivatives with 41 chromosomes approximated a 3:2 ratio. From these reports it appears that such ratios may vary widely, depending on the species or hybrid concerned. Possibly, too, environmental conditions may cause marked deviations in the ratio of n:(n−1) gametes.

SUMMARY

Seven monosomic (2n−1) types of *N. tabacum* var. *purpurea* are described. With the exception of haplo-F, all were obtained by interspecific hybridization and repeated backcrossing of the more fertile derivatives to the normal strain.

Characteristic of the types studied is a slight reduction in the vigor of growth accompanying a retardation in the rate of development.

All the types are markedly less fertile than the normal *purpurea* strain.

The pollen of each monosomic type except, possibly, haplo-A, is dimorphic.

The transmission of the monosomic condition through ovules is high, ranging from 45.6 percent in haplo-N to 83.2 percent in haplo-A. If seed viability and ultimate survival of the plants be considered, the number of functional (n−1) eggs is always well above 50 percent.

The highest transmission of the monosomic condition through the pollen was recorded in haplo-N, where only 7 percent of the progeny were mono-

somic. In the cross normal ♀ ×haplo-C♂ no monosomic plants were recovered.

Evidence is presented which indicates that univalent elimination may occur more frequently during megasporogenesis than microsporogenesis.

There appears to be no correlation between the transmission of the monosomic condition through the eggs and pollen of the same haplo-type.

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LITERATURE CITED

AVERY, P., 1929 Chromosome number and morphology in Nicotiana. IV. The nature and effects of chromosomal irregularities in *N. alata* var. *grandiflora*. Univ. California Pub. Bot. **11**: 265–284.

CLAUSEN, R. E., 1930 Inheritance in *Nicotiana tabacum*. X. Carmine-coral variegation. Cytologia **1**: 358–368.

1931 Inheritance in *Nicotiana tabacum*. XI. The fluted assemblage. Amer. Nat. **65**: 316–331.

CLAUSEN, R. E. and GOODSPEED, T. H., 1926a Inheritance in *Nicotiana tabacum*. VII. The monosomic character "fluted." Univ. California Pub. Bot. **11**: 61–82.

1926b Interspecific hybridization in *Nicotiana*. III. The monosomic *tabacum* derivative "corrugated" from the *sylvestris-tabacum* hybrid. Univ. California Pub. Bot. **11**: 83–101.

GOODSPEED, T. H. and AVERY, P., 1933 Inheritance in *Nicotiana tabacum*. XIII. The cytogenetics of "deformed," an X-ray derivative. Genetics **18**: 487–521.

GOODSPEED, T. H. and CLAUSEN, R. E., 1922 Interspecific hybridization in *Nicotiana*. I. On the results of backcrossing the F_1 *sylvestris-tabacum* hybrids to *sylvestris*. Univ. California Pub. Bot. **11**: 1–30.

1928 Interspecific hybridization in *Nicotiana*. VIII. The *sylvestris-tomentosa-tabacum* hybrid triangle and its bearing on the origin of tabacum. Univ. California Pub. Bot. **11**: 245–256.

HAKANSSON, ARTUR, 1932 Zytologische Studien an compactoiden Typen von *Triticum vulgare*. Hereditas **17**: 155–196.

HUSKINS, C. L., 1928 On the cytology of speltoid wheats in relation to their origin and genetic behavior. J. Genet. **20**: 103–122.

KIHARA, H., 1924 Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. **1**: 1–200.

1932 Chromosomale Aberranten bei *Pharbitis Nil*. Kwagaku **2**: 196–198.

LAMMERTS, W. E., 1932 Inheritance of monosomics in *Nicotiana rustica*. Genetics **17**: 689–696.

LI, JU-CHI, 1927 The effect of chromosome aberrations on development in *Drosophila melanogaster*. Genetics **12**: 1–58.

NISHIYAMA, I., 1928 On hybrids between *Triticum spelta* and two dwarf wheat plants with 40 somatic chromosomes. Bot. Mag. Tokyo **42**: 154–177.

1931 Genetics and cytology of certain cereals. II. Karyogenetic studies of fatuoid oats with special references to their origin. Jap. J. Genet. **7**: 49–102.

1933 The genetics and cytology of certain cereals. IV. Further studies on fatuoid oats. Jap. J. Genet. **8**: 107–124.

OLMO, H. P., 1935 Cytological studies of monosomic and derivative types of *Nicotiana tabacum*. Cytologia, in press.

POWERS, LEROY, 1932 Cytologic and genetic studies of variability of strains of wheat derived from interspecific crosses. J. Agric. Res. **44**: 797–831.